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(54) **Estrogen receptor**

Östrogen-Rezeptor

Récepteur d'oestrogène

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Description

[0001] This invention relates to the field of receptors belonging to the superfamily of nuclear hormone receptors, in particular to steroid receptors. The invention relates to DNA encoding a novel steroid receptor, the preparation of said receptor, the receptor protein, and the uses thereof.

[0002] Steroid hormone receptors belong to a superfamily of nuclear hormone receptors involved in ligand-dependent transcriptional control of gene expression. In addition, this superfamily consists of receptors for non-steroid hormones such as vitamin D, thyroid hormones and retinoids (Giguère et al, Nature 330, 624-629, 1987; Evans, R.M., Science 240, 889-895, 1988). Moreover, a range of nuclear receptor-like sequences have been identified which encode so-called 'orphan' receptors: these receptors are structurally related to and therefore classified as nuclear receptors, although no putative ligands have been identified yet (B.W. O'Malley, Endocrinology 125, 1119-1170, 1989; D.J. Mangelsdorf and R.M. Evans, Cell, 83, 841-850, 1995).

[0003] The superfamily of nuclear hormone receptors share a modular structure in which six distinct structural and functional domains, A to F, are displayed (Evans, Science 240, 889-895, 1988). A nuclear hormone receptor is characterized by a variable N-terminal region (domain A/B), followed by a centrally located, highly conserved DNA-binding domain (hereinafter referred to as DBD; domain C), a variable hinge region (domain D), a conserved ligand-binding domain (herein after referred to as LBD; domain E) and a variable C-terminal region (domain F).

[0004] The N-terminal region, which is highly variable in size and sequence, is poorly conserved among the different members of the superfamily. This part of the receptor is involved in the modulation of transcription activation (Bocquel et al, Nucl. Acid Res., 17, 2581-2595, 1989; Tora et al, Cell 59, 477-487, 1989).

[0005] The DBD consists of approximately 66 to 70 amino acids and is responsible for DNA-binding activity: it targets the receptor to specific DNA sequences called hormone responsive elements (hereinafter referred to as HRE) within the transcription control unit of specific target genes on the chromatin (Martinez and Wahli, In 'Nuclear Hormone Receptors', Acad. Press, 125-153, 1991).

[0006] The LBD is located in the C-terminal part of the receptor and is primarily responsible for ligand binding activity. In this way, the LBD is essential for recognition and binding of the hormone ligand and, in addition possesses a transcription activation function, thereby determining the specificity and selectivity of the hormone response of the receptor. Although moderately conserved in structure, the LBD's are known to vary considerably in homology between the individual members of the nuclear hormone receptor superfamily (Evans, Science 240, 889-895, 1988; P.J. Fuller, FASEB J., 5, 3092-3099, 1991; Mangelsdorf et al, Cell, Vol. 83, 835-839, 1995).

[0007] Functions present in the N-terminal region, LBD and DBD operate independently from each other and it has been shown that these domains can be exchanged between nuclear receptors (Green et al, Nature, Vol. 325, 75-78, 1987). This results in chimeric nuclear receptors, such as described for instance in WO-A-8905355.

[0008] When a hormone ligand for a nuclear receptor enters the cell by diffusion and is recognized by the LBD, it will bind to the specific receptor protein, thereby initiating an allosteric alteration of the receptor protein. As a result of this alteration the ligand/receptor complex switches to a transcriptionally active state and as such is able to bind through the presence of the DBD with high affinity to the corresponding HRE on the chromatin DNA (Martinez and Wahli, 'Nuclear Hormone Receptors', 125-153, Acad. Press, 1991). In this way the ligand/receptor complex modulates expression of the specific target genes. The diversity achieved by this family of receptors results from their ability to respond to different ligands.

[0009] The steroid hormone receptors are a distinct class of the nuclear receptor superfamily, characterized in that the ligands are steroid hormones. The receptors for glucocorticoids (GR), mineralcorticoids (MR), progestins (PR), androgens (AR) and estrogens (ER) are classical steroid receptors. Furthermore, the steroid receptors have the unique ability upon activation to bind to palindromic DNA sequences, the so-called HRE's, as homodimers. The GR, MR, PR and AR recognize the same DNA sequence, while the ER recognizes a different DNA sequence. (Beato et al, Cell, Vol. 83, 851-857, 1995). After binding to DNA, the steroid receptor is thought to interact with components of the basal transcriptional machinery and with sequence-specific transcription factors, thus modulating the expression of specific target genes.

[0010] Several HRE's have been identified, which are responsive to the hormone/receptor complex. These HRE's are situated in the transcriptional control units of the various target genes such as mammalian growth hormone genes (responsive to glucocorticoid, estrogen, testosterone), mammalian prolactin genes and progesterone receptor genes (responsive to Estrogen), avian ovalbumin genes (responsive to progesterone), mammalian metallothionein gene (responsive to glucocorticoid) and mammalian hepatic α_{2u} -globulin gene (responsive to estrogen, testosterone, glucocorticoid).

[0011] The steroid hormone receptors have been known to be involved in embryonic development, adult homeostasis as well as organ physiology. Various diseases and abnormalities have been ascribed to a disturbance in the steroid hormone pathway. Since the steroid receptors exercise their influence as hormone-activated transcriptional modulators, it can be anticipated that mutations and defects in these receptors, as well as overstimulation or blocking of these

receptors might be the underlying reason for the altered pattern. A better knowledge of these receptors, their mechanism of action and of the ligands which bind to said receptor might help to create a better insight in the underlying mechanism of the hormone signal transduction pathway, which eventually will lead to better treatment of the diseases and abnormalities linked to altered hormone/receptor functioning.

5 **[0012]** For this reason cDNA's of the steroid and several other nuclear receptors of several mammals, including humans, have been isolated and the corresponding amino acid sequences have been deduced, such as for example the human steroid receptors PR, ER, GR, MR, and AR, the human non-steroid receptors for vitamin D, thyroid hormones, and retinoids such as retinol A and retinoic acid. In addition, cDNA's encoding well over 100 mammalian orphan receptors have been isolated, for which no putative ligands are known yet (Mangelsdorf et al, Cell, Vol.83, 835-839, 10 1995). However, there is still a great need for the elucidation of other nuclear receptors in order to unravel the various roles these receptors play in normal physiology and pathology.

[0013] The present invention provides for such a novel nuclear receptor. More specifically, the invention provides for an isolated estrogen receptor having an N-terminal domain, a DNA-binding domain, and a ligand-binding domain, wherein the amino acid sequence of said DNA-binding domain exhibits at least 80% homology with the amino acid sequence shown in SEQ ID NO:3 and the amino acid sequence of said ligand-binding domain of said estrogen receptor exhibits at least 70 % homology with the amino acid sequence shown in SEQ ID NO:4, 15 provided that the estrogen receptor does not have the amino acid sequence:

20 MTFYS PAVMN YSVPG STSNL DGGPV RLSTS PNVLW PTSGH LSPLA
 THCQS SLLYA EPQKS PWCEA RSLEH TLPVN RETLK RKLSG SSCAS
 PVTSP NAKRD AHFCP VCS DY ASGYH YGVWS CEGCK AFFKR SIQGH
 NDYIC PATNQ CTIDK NRRKS CQACR LRKCY EVGMV KCGSR RERCG
 25 YRIVR RQRSS SEQVH CLSKA KRNGG HAPRV KELL L STLSP EQLVL
 TLLEA EPPNV LVS RP SMPFT EASMM MSLTK LADKE LVHMI GWAKK
 IPGFV ELSLL DQVRL LESCW MEVLM VGLMW RSIDH PGKLI FAPDL
 VLDRD EGKCV EGILE IFDML LATT S RFREL KLQHK EYLCV KAMIL
 30 LNSSM YPLAS ANQEA ESSRK LTHLL NAVTD ALVWV IAKSG ISSQQ
 QSVRL ANLLM LLSHV RHISN KGMEH LLSMK CKNVV PVYDL LLEML
 NAHTL RGYKS SISGS ECSST EDSKN KESSQ NLQSQ.

35 The disclaimer relates to the non-prepublished patent application WO 97/09348.

[0014] Hereby, the present invention provides for a novel steroid receptor, having estrogen mediated activity. Said novel steroid receptors are novel estrogen receptors, which are able to bind and be activated by, for example, estradiol, estrone and estriol. According to the present invention it has been found that a novel estrogen receptor is expressed as an 8 kb transcript in human thymus, spleen, peripheral blood lymphocytes (PBLs), ovary and testis. Furthermore, 40 additional transcripts have been identified. Another transcript of approximately 10 kb was identified in ovary, thymus and spleen. In testis, an additional transcript of 1.3 kb was detected. These transcripts are probably generated by alternative splicing of the gene encoding the novel estrogen receptor according to the invention.

[0015] Cloning of the cDNA's encoding the novel estrogen receptors according to the invention revealed that several splicing variants of said receptor can be distinguished. At the protein level, these variants differ only at the C-terminal part. 45

[0016] cDNA encoding an ER has been isolated (Green, et al, Nature 320, 134-139, 1986; Greene et al, Science 231, 1150-1154, 1986), and the corresponding amino acid sequence has been deduced. This receptor and the receptor according to the present invention, however, are distinct, and encoded for by different genes with different nucleic acid sequences. Not only do the ER of the prior art (hereinafter referred to as classical ER) and the ER according to the present invention differ in amino acid sequence, they also are located on different chromosomes. The gene encoding the classical ER is located on chromosome 6, whereas the gene encoding the ER according to the invention was found to be located on chromosome 14. The ER according to the invention furthermore distinguishes itself from the classical receptor in differences in tissue distribution, indicating that there may be important differences between these receptors at the level of estrogenic signalling. 50 55

[0017] In addition, two orphan receptors, ERR α and ERRS, having an estrogen receptor related structure have been described (Giguère et al, Nature 331, 91-94, 1988). These orphan receptors, however, have not been reported to be able to bind estradiol or any other hormone that binds to the classical ER, and other ligands which bind to these

receptors have not been found yet. The novel estrogen receptor according to the invention distinguishes itself clearly from these receptors since it was found to bind estrogens.

5 [0018] The fact that a novel ER according to the invention has been found is all the more surprising, since any suggestion towards the existence of additional estrogen receptors was absent in the scientific literature: neither the isolation of the classical ER nor the orphan receptors $ERR\alpha$ and $ERR\beta$ suggested or hinted towards the presence of additional estrogen receptors such as the receptors according to the invention. The identification of additional ER's could be a major step forward for the existing clinical therapies, which are based on the existence of one ER and as such ascribe all estrogen mediated abnormalities and/or diseases to this one receptor. The receptors according to the invention will be useful in the development of hormone analogs that selectively activate either the classical ER or the novel estrogen receptor according to the invention. This should be considered as one of the major advantages of the present invention.

[0019] Thus, in one aspect, the present invention provides for isolated cDNA encoding a novel steroid receptor. In particular, the present invention provides for isolated cDNA encoding the novel estrogen receptor as defined above.

15 [0020] According to this aspect of the present invention, there is provided an isolated DNA encoding a steroid receptor protein having an N-terminal domain, a DNA-binding domain and a ligand-binding domain, wherein the amino acid sequence of said DNA-binding domain of said receptor protein exhibits at least 80% homology with the amino acid sequence shown in SEQ ID NO:3, and the amino acid sequence of said ligand-binding domain of said receptor protein exhibits at least 70% homology with the amino acid sequence shown in SEQ ID NO:4.

20 [0021] In particular, the isolated DNA encodes a steroid receptor protein having an N-terminal domain, a DNA-binding domain and a ligand-binding domain, wherein the amino acid sequence of said DNA-binding domain of said receptor protein exhibits at least 90%, preferably 95%, more preferably 98%, most preferably 100% homology with the amino acid sequence shown in SEQ ID NO:3.

25 [0022] More particularly, the isolated DNA encodes a steroid receptor protein having an N-terminal domain, a DNA-binding domain and a ligand-binding domain, wherein the amino acid sequence of said ligand-binding domain of said receptor protein exhibits at least 75%, preferably 80%, more preferably 90%, most preferably 100% homology with the amino acid sequence shown in SEQ ID NO:4.

[0023] A preferred isolated DNA according to the invention encodes a steroid receptor protein having the amino acid sequence shown in SEQ ID NO:5, SEQ ID NO:6, SEQ ID NO:21 or SEQ ID NO:25.

30 [0024] A more preferred isolated DNA according to the invention is an isolated DNA comprising a nucleotide sequence shown in SEQ ID NO:1, SEQ ID NO:2, SEQ ID NO:20 or SEQ ID NO:24.

[0025] The DNA according to the invention may be obtained from cDNA. Alternatively, the coding sequence might be genomic DNA, or prepared using DNA synthesis techniques.

[0026] The DNA according to the invention will be very useful for in vivo expression of the novel receptor proteins according to the invention in sufficient quantities and in substantially pure form.

35 [0027] In another aspect of the invention, there is provided for a steroid receptor comprising the amino acid sequence encoded by the above described DNA molecules.

[0028] The steroid receptor according to the invention has an N-terminal domain, a DNA-binding domain and a ligand-binding domain, wherein the amino acid sequence of said DNA-binding domain of said receptor exhibits at least 80% homology with the amino acid sequence shown in SEQ ID NO:3, and the amino acid sequence of said ligand-binding domain of said receptor exhibits at least 70% homology with the amino acid sequence shown in SEQ ID NO:4.

40 [0029] In particular, the steroid receptor according to the invention has an N-terminal domain, a DNA-binding domain and a ligand-binding domain, wherein the amino acid sequence of said DNA-binding domain of said receptor exhibits at least 90%, preferably 95%, more preferably 98%, most preferably 100% homology with the amino acid sequence shown in SEQ ID NO:3.

45 [0030] More particular, the steroid receptor according to the invention has an N-terminal domain, a DNA-binding domain and a ligand-binding domain, wherein the amino acid sequence of said ligand-binding domain of said receptor exhibits at least 75%, preferably 80%, more preferably 90%, most preferably 100% homology with the amino acid sequence shown in SEQ ID NO:4.

50 [0031] It will be clear for those skilled in the art that also steroid receptor proteins comprising combined DBD and LBD preferences and DNA encoding such receptors are subject of the invention.

[0032] Preferably, the steroid receptor according to the invention comprises an amino acid sequence shown in SEQ ID NO:5, SEQ ID NO:6, SEQ ID NO:21 or SEQ ID NO:25.

55 [0033] Also within the scope of the present invention are steroid receptor proteins which comprise variations in the amino acid sequence of the DBD and LBD without losing their respective DNA-binding or ligand-binding activities. The variations that can occur in those amino acid sequences comprise deletions, substitutions, insertions, inversions or additions of (an) amino acid(s) in said sequence, said variations resulting in amino acid difference(s) in the overall sequence. It is well known in the art of proteins and peptides that these amino acid differences lead to amino acid sequences that are different from, but still homologous with the native amino acid sequence they have been derived

from.

[0034] Amino acid substitutions that are expected not to essentially alter biological and immunological activities, have been described in for example Dayhof, M.D., Atlas of protein sequence and structure, Nat. Biomed. Res. Found., Washington D.C., 1978, vol. 5, suppl. 3. Amino acid replacements between related amino acids or replacements which have occurred frequently in evolution are, inter alia Ser/Ala, Ser/Gly, Asp/Gly, Arg/Lys, Asp/Asn, Ile/Val. Based on this information Lipman and Pearson developed a method for rapid and sensitive protein comparison (Science 227, 1435-1441, 1985) and determining the functional similarity between homologous polypeptides.

[0035] Variations in amino acid sequence of the DBD according to the invention resulting in an amino acid sequence that has at least 80% homology with the sequence of SEQ ID NO:3 will lead to receptors still having sufficient DNA binding activity. Variations in amino acid sequence of the LBD according to the invention resulting in an amino acid sequence that has at least 70% homology with the sequence of SEQ ID NO:4 will lead to receptors still having sufficient ligand binding activity.

[0036] Homology as defined herein is expressed in percentages, determined via PCGENE. Homology is calculated as the percentage of identical residues in an alignment with the sequence according to the invention. Gaps are allowed to obtain maximum alignment.

[0037] Comparing the amino acid sequences of the classical ER and the ER's according to the invention revealed a high degree of similarity within their respective DBD's. The conservation of the P-box (amino acids E-G-X-X-A) which is responsible for the actual interactions of the classical ER with the target DNA element (Zilliaccus et al., Mol.Endo. 9, 389, 1995; Glass, End.Rev. 15, 391, 1994), is indicative for a recognition of estrogen responsive elements (ERE's) by the ER's according to the invention. The receptors according to the invention indeed showed ligand-dependent trans-activation on ERE-containing reporter constructs. Therefore, the classical ER and the novel ER's according to the invention may have overlapping target gene specificities. This could indicate that in tissues which co-express both respective ER's, these receptors compete for ERE's. The ER's according to the invention may regulate transcription of target genes differently from classical ER regulation or could simply block classical ER functioning by occupying estrogen responsive elements. Alternatively, transcription might be influenced by heterodimerization of the different receptors.

[0038] Thus, a preferred steroid receptor according to the invention comprises the amino acid sequence E-G-X-X-A within the P box of the DNA binding domain, wherein X stands for any amino acid. Also within the scope of the invention is isolated DNA encoding such a receptor.

[0039] Methods to prepare the receptors according to the invention are well known in the art (Sambrook et al., Molecular Cloning: a Laboratory Manual, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, 1989). The most practical approach is to produce these receptors by expression of the DNA encoding the desired protein.

[0040] A wide variety of host cell and cloning vehicle combinations may be usefully employed in cloning the nucleic acid sequence coding for the receptor of the invention. For example, useful cloning vehicles may include chromosomal, non-chromosomal and synthetic DNA sequences such as various known bacterial plasmids and wider host range plasmids and vectors derived from combinations of plasmids and phage or virus DNA. Useful hosts may include bacterial hosts, yeasts and other fungi, plant or animal hosts, such as Chinese Hamster Ovary (CHO) cells or monkey cells and other hosts (with exception of linman beings).

[0041] Vehicles for use in expression of the ligand-binding domain of the present invention will further comprise control sequences operably linked to the nucleic acid sequence coding for the ligand-binding domain. Such control sequences generally comprise a promoter sequence and sequences which regulate and/or enhance expression levels. Furthermore an origin of replication and/or a dominant selection marker are often present in such vehicles. Of course control and other sequences can vary depending on the host cell selected.

[0042] Techniques for transforming or transfecting host cells are quite known in the art (see, for instance, Sambrook et al., Molecular Cloning: A Laboratory Manual, Cold Spring Harbor Laboratory, 1989).

[0043] Recombinant expression vectors comprising the DNA of the invention as well as cells transformed with said DNA or said expression vector also form part of the present invention.

[0044] Since steroid receptors have three domains with different functions, which are more or less independent, it is possible that all three functional domains have been derived from different members of the steroid receptor super-family.

[0045] Molecules which contain parts having a different origin are called chimeric. Such a chimeric receptor comprising the ligand-binding domain and/or the DNA-binding domain of the invention may be produced by chemical linkage, but most preferably the coupling is accomplished at the DNA level with standard molecular biological methods by fusing the nucleic acid sequences encoding the necessary steroid receptor domains. Hence, DNA encoding the chimeric receptor proteins according to the invention are also subject of the present invention.

[0046] Such chimeric proteins can be prepared by transfecting DNA encoding these chimeric receptor proteins to suitable host cells and culturing these cells under suitable conditions.

[0047] It is extremely practical if, next to the information for the expression of the steroid receptor, also the host cell

is transformed or transfected with a vector which carries the information for a reporter molecule. Such a vector coding for a reporter molecule is characterized by having a promoter sequence containing one or more hormone responsive elements (HRE) functionally linked to an operative reporter gene. Such a HRE is the DNA target of the activated steroid receptor and, as a consequence, it enhances the transcription of the DNA coding for the reporter molecule. In *in vivo* settings of steroid receptors the reporter molecule comprises the cellular response to the stimulation of the ligand. However, it is possible *in vitro* to combine the ligand-binding domain of a receptor to the DNA binding domain and transcription activating domain of other steroid receptors, thereby enabling the use of other HRE and reporter molecule systems. One such a system is established by a HRE presented in the MMTV-LTR (mouse mammary tumor virus long terminal repeat sequence in connection with a reporter molecule like the firefly luciferase gene or the bacterial gene for CAT (chloramphenicol transferase). Other HRE's which can be used are the rat oxytocin promoter, the retinoic acid responsive element, the thyroid hormone responsive element, the estrogen responsive element and also synthetic responsive elements have been described (for instance in Fuller, *ibid.* page 3096). As reporter molecules next to CAT and luciferase β -galactosidase can be used.

[0048] Steroid hormone receptors and chimeric receptors according to the present invention can be used for the *in vitro* identification of novel ligands or hormonal analogs. For this purpose binding studies can be performed with cells transformed with DNA according to the invention or an expression vector comprising DNA according to the invention, said cells expressing the steroid receptors or chimeric receptors according to the invention.

[0049] The novel steroid hormone receptor and chimeric receptors according to the invention as well as the ligand-binding domain of the invention, can be used in an assay for the identification of functional ligands or hormone analogs for the nuclear receptors.

[0050] Thus, the present invention provides for a method for identifying functional ligands for the steroid receptors and chimeric receptors according to the invention, said method comprising the steps of

- a) introducing into a suitable host cell 1) DNA or an expression vector according to the invention, and 2) a suitable reporter gene functionally linked to an operative hormone response element, said HRE being able to be activated by the DNA-binding domain of the receptor protein encoded by said DNA;
- b) bringing the host cell from step a) into contact with potential ligands which will possibly bind to the ligand-binding domain of the receptor protein encoded by said DNA from step a);
- c) monitoring the expression of the receptor protein encoded by said reporter gene of step a).

[0051] If expression of the reporter gene is induced with respect to basic expression (without ligand), the functional ligand can be considered as an agonist; if expression of the reporter gene remains unchanged or is reduced with respect to basic expression, the functional ligand can be a suitable (partial) antagonist.

[0052] For performing such kind of investigations host cells which have been transformed or transfected with both a vector encoding a functional steroid receptor and a vector having the information for a hormone responsive element and a connected reporter molecule are cultured in a suitable medium. After addition of a suitable ligand, which will activate the receptor the production of the reporter molecule will be enhanced, which production simply can be determined by assays having a sensitivity for the reporter molecule. See for instance WO-A-8803168. Assays with known steroid receptors have been described (for instance S. Tsai et al., *Cell* 57, 443, 1989; M. Meyer et al., *Cell* 57, 433, 1989).

Legends to the figures

Figure 1.

[0053] Northern analysis of the novel estrogen receptor (ER β). Two different multiple tissue Northern blots (Clontech) were hybridised with a specific probe for ER β (see examples). Indicated are the human tissues the RNA originated from and the position of the size markers in kilobases (kb).

Figure 2.

[0054] Histogram showing the 3- to 4-fold stimulatory effect of 17 β -estradiol, estriol and estrone on the luciferase activity mediated by ER β . An expression vector encoding ER β was transiently transfected into CHO cells together with a reporter construct containing the rat oxytocin promoter in front of the firefly luciferase encoding sequence (see examples).

Figure 3.

[0055] Effect of 17 β -estradiol (E2) alone or in combination with the anti-estrogen ICI-164384 (ICI) on ER α and ER β .

Expression constructs for ER α (the classical ER) and ER β were transiently transfected into CHO cells together with the rat oxytocin promoter-luciferase reporter construct described in the examples. Luciferase activities were determined in triplicate and normalised for transfection efficiency by measuring β -galactosidase in the same lysate.

5 **Figure 4.**

[0056] Expression of ER α and ER β in a number of cell lines determined by RT-PCR analysis (see examples). The cell lines used were derived from different tissues/cell types: endometrium (ECC1, Ishikawa, HEC-1A, RL95-2); osteosarcoma (SAOS-2, U2-OS, HOS, MG63); breast tumours (MCF-7, T47D), endothelium (HUV-EC-C, BAEC-1); smooth muscle (HISM, PAC-1, A7R5, A10, RASMC, CavaSMC); liver (HepG2); colon (CaCo2); and vagina (Hs-760T, SW-954).

[0057] All cell lines were human except for PAC-1, A7R5, A10 and RASMC which are of rat origin, BAEC-1 which is of bovine origin and CavaSMC which is of guinea pig origin.

15 **Figure 5.**

[0058] Transactivation assay using stably transfected CHO cell lines expressing ER α or ER β together with the rat oxytocin-luciferase estrogen-responsive reporter (see examples for details). Hormone-dependent transactivation curves were determined for 17 β -estradiol and for Org4094. For the ER antagonist raloxifen, cells were treated with 2 x 10⁻¹⁰ mol/L 17 β -estradiol together with increasing concentrations of raloxifen. Maximal values of the responses were arbitrarily set at 100%.

20 **Examples**

25 **A. Molecular cloning of the novel estrogen receptor.**

[0059] Two degenerate oligonucleotides containing inosines (I) were based on conserved regions of the DNA-binding domains and the ligand-binding domains of the human steroid hormone receptors.

[0060] Primer #1:

30 5' -GGIGA (C/T) GA (A/G) GC (A/T) TCIGGITG (C/T) CA (C/T) TA (C/T) GG-3'

(SEQ ID NO:7).

[0061] Primer #2:

35 5' -AAGCCTGG (C/G) A (C/T) IC (G/T) (C/T) TTIGCCCAI (C/T) TIAT-3'

(SEQ ID NO:8).

40 [0062] As template, cDNA from human EBV-stimulated PBLs (peripheral blood leukocytes) was used. One microgram of total RNA was reverse transcribed in a 20 μ l reaction containing 50 mM KCl, 10 mM Tris-HCl pH 8.3, 4 mM MgCl₂, 1 mM dNTPs (Pharmacia), 100 pmol random hexanucleotides (Pharmacia), 30 Units RNase inhibitor (Pharmacia) and 200 Units M-MLV Reverse transcriptase (Gibco BRL). Reaction mixtures were incubated at 37°C for 30 minutes and heat-inactivated at 100°C for 5 minutes. The cDNA obtained was used in a 100 μ l PCR reaction containing 10 mM Tris-HCl pH 8.3, 50 mM KCl, 1.5 mM MgCl₂, 0.001% gelatin (w/v), 3% DMSO, 1 microgram of primer #1 and primer #2 and 2.5 Units of Amplitaq DNA polymerase (Perkin Elmer). PCR reactions were performed in the Perkin Elmer 9600 thermal cycler. The initial denaturation (4 minutes at 94°C) was followed by 35 cycles with the following conditions: 30 sec. 94°C, 30 sec. 45°C, 1 minute 72°C and after 7 minutes at 72°C the reactions were stored at 4°C. Aliquots of these reactions were analysed on a 1.5% agarose gel. Fragments of interest were cut out of the gel, reamplified using identical PCR-conditions and purified using Qiaex II (Qiagen). Fragments were cloned in the pCRII vector and transformed into bacteria using the TA-cloning kit (Invitrogen). Plasmid DNA was isolated for nucleotide sequence analysis using the Qiagen plasmid midi protocol (Qiagen). Nucleotide sequence analysis was performed with the ALF automatic sequencer (Pharmacia) using a T7 DNA sequencing kit (Pharmacia) with vector-specific or fragment-specific primers.

55 [0063] One cloned fragment corresponded to a novel estrogen receptor (ER) which is closely related to the classical estrogen receptor. Part of the cloned novel estrogen receptor fragment (nucleotides 466 to 797 in SEQ ID 1) was amplified by PCR using oligonucleotide #3 TGTTACGAAGTGGGAATGGTGA (SEQ ID NO:9) and oligonucleotide #2 and used as a probe to screen a human testis cDNA library in λ gt11 (Clontech #HL1010b). Recombinant phages were

plated (using Y1090 bacteria grown in LB medium supplemented with 0.2% maltose) at a density of 40.000 pfu (plaque-forming units) per 135 mm dish and replica filters (Hybond-N, Amersham) were made as described by the supplier. Filters were prehybridised in a solution containing 0.5 M phosphate buffer (pH 7.5) and 7% SDS at 65°C for at least 30 minutes. DNA probes were purified with Qiaex II (Qiagen), ³²P-labeled with a Decaprime kit (Ambion) and added to the prehybridisation solution. Filters were hybridised at 65°C overnight and then washed in 0.5 X SSC/0.1% SDS at 65°C. Two positive plaques were identified and could be shown to be identical. These clones were purified by rescreening one more time. A PCR reaction on the phage eluates with the λgt11-specific primers #4: 5'-TTGACACCA-GACCAACTGGTAATG-3' (SEQ ID NO:10) and #5: 5'-GGTGGCGACGACTCCTGGAGCCCG-3' (SEQ ID NO:11) yielded a fragment of 1700 basepairs on both clones. Subsequent PCR reactions using combinations of a gene-specific primer #6: 5'-GTACACTGATTTGTAGCTGGAC-3' (SEQ ID NO:12) with the λgt11 primer #4 and gene-specific primer #7: 5'-CCATGATGATGTCCTGACC-3' (SEQ ID NO:13) with λgt11 primer #5 yielded fragments of approximately 450 bp and 1000 bp, respectively, which were cloned in the pCRII vector and used for nucleotide sequence analysis. The conditions for these PCR reactions were as described above except for the primer concentrations (200 ng of each primer) and the annealing temperature (60°C). Since in the cDNA clone the homology with the ER is lost abruptly at a site which corresponds to the exon 7/exon 8 boundary in the ER (between nucleotides 1247 and 1248 in SEQ ID NO:1), it was suggested that this sequence corresponds to intron 7 of the novel ER gene. For verification of the nucleotide sequences of this cDNA clone, a 1200 bp fragment was generated on the cDNA clone with λgt11 primer #4 with a gene-specific primer #8 corresponding to the 3' end of exon 7: 5'-TCGCATGCCTGACGTGGGAC-3' (SEQ ID NO:14) using the proofreading *Pfu* polymerase (Stratagene). This fragment was also cloned in the pCRII vector and completely sequenced and was shown to be identical to the sequences obtained earlier.

[0064] To obtain nucleotide sequences of the novel ER downstream of exon 7, a degenerate oligonucleotide based on the AF-2 region of the classical ER (#9: 5'-GGC(C/G)TCCAGCATCTCCAG(C/G)A(A/G)CAG-3'; SEQ ID NO:15) was used together with the gene-specific oligonucleotide #10: 5'-GGAAGCTGGCTCACTTGCTG-3' (SEQ ID NO:16) using testis cDNA as template (Marathon ready testis cDNA, Clontech Cat #7414-1). A specific 220 bp fragment corresponding to nucleotides 1112 to 1332 in SEQ ID No. 1 was cloned and sequenced. Nucleotides 1112 to 1247 were identical to the corresponding sequence of the cDNA clone. The sequence downstream thereof is highly homologous with the corresponding region in the classical ER. In order to obtain sequences of the novel ER downstream of the AF-2 region, RACE (rapid amplification of cDNA ends) PCR reactions were performed using the Marathon-ready testis cDNA (Clontech) as template. The initial PCR was performed using oligonucleotide #11: 5'-TCTTGTCTGGACAG-GGATG-3' (SEQ ID NO:17) in combination with the AP1 primer provided in the kit. A nested PCR was performed on an aliquot of this reaction using oligonucleotide #10 (SEQ ID NO:16) in combination with the oligo dT primer provided in the kit. Subsequently, an aliquot of this reaction was used in a nested PCR using oligonucleotide #12: 5'-GCATGGAA-CATCTGCTCAAC-3' (SEQ ID NO:18) in combination with the oligo dT primer. Nucleotide sequence analysis of a specific fragment that was obtained (corresponding to nucleotides 1256 to 1431 in SEQ ID NO 1) revealed a sequence encoding the carboxyterminus of the novel ER ligand-binding domain, including an F-domain and a translational stop codon and part of the 3' untranslated sequence which is not included in SEQ ID NO:1. The deduced amino acid sequence is shown in SEQ ID NO:5.

[0065] In order to investigate the possibility that the novel estrogen receptor had additional, upstream translation-initiation codons, RACE-PCR experiments were performed using Marathon-ready testis cDNA (Clontech Cat. # 7414-1). First a PCR was performed using oligonucleotide SEQ ID NO:12 (antisense corresponding to nucleotides 416-395 in SEQ ID NO:1) and AP-1 (provided in the kit). A nested PCR was then performed using oligonucleotide having SEQ ID NO:27 (antisense corresponding to nucleotides 254-231 in SEQ ID NO:1) with AP-2 (provided in the kit). From the smear that was obtained, the region corresponding to fragments larger than 300 basepairs was cut out, purified using the GeneCleanII kit (Bio101) and cloned using the TA-cloning kit (Clontech). Colonies were screened by PCR using gene-specific primers: SEQ ID NO:22 and SEQ ID NO:28. The clone containing the largest insert was sequenced. The nucleotide sequence corresponds to nucleotides 1 to 490 in SEQ ID NO:24. It is clear from this sequence that the first in-frame upstream translation initiation codon is present at position 77-79 in SEQ ID NO:24. Upstream of this translational startcodon an in-frame stop-codon is present (11-13 in SEQ ID NO:24). Consequently, the reading frame of the novel estrogen receptor is 530 amino acids (shown in SEQ ID NO:25) and has a calculated molecular mass of 59.234 kD.

[0066] To confirm the nucleotide sequences obtained by 5' RACE, human genomic clones were obtained and analysed. A human genomic library in λEMBL3 (Clontech HL1067J) was screened with a probe corresponding to nucleotides 1 to 416 in SEQ ID NO:1. A strongly hybridizing clone was plaque-purified and DNA was isolated using standard protocols (Sambrook et al, 1989). The DNA was digested with several restriction enzymes, electrophoresed on agarose gel and blotted onto Nylon filters. Hybridisation of the blot with a probe corresponding to the above-mentioned RACE fragment (nucleotides 1-490 in SEQ ID NO:24) revealed a hybridizing *Sau*3A fragment of approximately 800 basepairs. This fragment was cloned into the *Bam*H1 site of pGEM3Z and sequenced. The nucleotide sequence contained one base difference which is probably a PCR-induced point mutation in the RACE fragment. Nucleotide 172 was a G residue

in the 5'RACE fragment, but an A residue in several independent genomic subclones.

B. Identification of two splice variants of the novel estrogen receptor.

5 **[0067]** Rescreening of the testis cDNA library with a probe corresponding to nucleotides 918 to 1246 in SEQ ID No. 1 yielded two hybridizing clones, the 3' end of which were amplified by PCR (gene-specific primer #10: 5'-GGAAGCT-GGCTCACTTGCTG-3' (SEQ ID NO:16) together with primer #4, SEQ ID NO:10), cloned and sequenced. One clone was shown to contain an alternative exon 8 (exon 8B) of the novel ER. In SEQ ID No. 2 the protein encoding part and the stopcodon of this splice variant are presented. As a consequence of the introduction of this exon through an alter-
10 native splicing reaction, the reading frame encoding the novel ER is immediately terminated, thereby creating a truncation of the carboxyterminus of the novel ER (SEQ ID NO:6).

[0068] Screening of a human thymus cDNA library (Clontech HL1074a) with the probe corresponding to nucleotides 918 to 1246 in SEQ ID No. 1, revealed another splice variant. The 3' end of one hybridizing clone was amplified using primer #10 (SEQ-ID NO:16) with the λ gt10-specific primer #13 5'-AGCAAGTTCAGCCTGTTAAGT-3' (SEQ ID NO:19),
15 cloned and sequenced. The obtained nucleotide sequence upstream of the exon 7/exon 8 boundary was identical to the clones identified earlier. However, an alternative exon 8 (exon 8C) was present at the 3' end encoding two C-terminal amino acids followed by a stop-codon. The nucleotide sequence of the protein-encoding part of this splice variant is shown in SEQ ID NO:20, the corresponding protein sequence is SEQ ID NO:21.

[0069] These two variants of the novel estrogen receptor do not contain the AF-2 region and therefore probably lack the ability to modulate transcription of target genes in a ligand-dependent fashion. However, the variants potentially could interfere with the functioning of the wild-type classical ER and/or the wild-type novel ER, either by heterodimerization or by occupying estrogen response elements or by interactions with other transcription factors. A mutant of the classical ER (ER1-530) has been described which closely resembles the two variants of the novel estrogen receptor described above. ER1-530 has been shown to behave as a dominant-negative receptor i.e. it can modulate the intra-
20 cellular activity of the wild type ER (Ince *et al*, J. Biol. Chem. 268, 14026-14032, 1993).
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C. Northern blot analysis.

[0070] Human multiple tissue Northern blots (MTN-blots) were purchased from Clontech and prehybridized for at least 1 hour at 65°C in 0.5 M phosphate buffer pH 7.5 with 7% SDS. The DNA fragment that was used as a probe (corresponding to nucleotides 466 to 797 in SEQ ID No. 1) was ³²P-labeled using a labelling kit (Ambion), denatured by boiling and added to the prehybridisation solution. Washing conditions were: 3X SSC at room temperature, followed by 3 X SSC at 65°C, and finally 1 X SSC at 65°C. The filters were then exposed to X-ray films for one week. Two transcripts of approximately 8 kb and 10 kb were detected in thymus, spleen, ovary and testis. In addition, a 1.3 kb transcript was detected in testis.
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D. RT-PCR analysis of expression of ER α and ER β in cell lines.

[0071] RNA was isolated from a number of human and animal cell lines using RNAzol B (Cinna/Biotech). cDNA was made using 2.5 microgram of total RNA using the Superscript II kit (BRL) following the manufacturers instructions. A portion of the cDNA was used for specific PCR amplifications of fragments corresponding either to mRNA encoding the ER or to the novel estrogen receptor. (It should be emphasized that the primers used are based on human and rat sequences, whereas some of the cell lines were not rat or human, see legend of Figure 4). Primers used were for ER α : sense 5'-GATGGGCTTACTGACCAACC-3' and antisense 5'-AGATGCTCCATGCCTTTG-3' generating a 548 base pair fragment corresponding to part of the LBD. For ER β : sense 5'- TTCACCGAGGCCTCCATGATG-3' and antisense 5'-CA-GATGTTCCATGCCCTTGTT-3' generating a 565 base pair fragment corresponding to part of the LBD. The PCR samples were analysed on agarose which were blotted onto Nylon membranes. These blots were hybridised with ³²P-labeled PCR fragments, generated with the above-mentioned primers on ER α and ER β plasmid DNA using standard experimental procedures (Sambrook *et al*, 1989).
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E. Ligand-dependent transcription activation by the novel estrogen receptor protein.

Cell culture

55 **[0072]** Chinese Hamster Ovary (CHO K1) cells were obtained from ATCC (CCL61) and maintained at 37°C in a humidified atmosphere (5% CO₂) as a monolayer culture in fenolred-free M505 medium. The latter medium consists of a mixture (1:1) of Dulbecco's Modified Eagle's Medium (DMEM, Gibco 074-200) and Nutrient Medium F12 (Ham's F12, Gibco 074-1700) supplemented with 2.5 mg/ml sodium carbonate (Baker), 55 μ g/ml sodium pyruvate (Fluka), 2.3

µg/ml β-mercaptoethanol (Baker), 1.2 µg/ml ethanolamine (Baker), 360 µg/ml L-glutamine (Merck), 0.45 µg/ml sodium selenite (Fluka), 62.5 µg/ml penicillin (Mycopharm), 62.5 µg/ml streptomycin (Serva), and 5% charcoal-treated bovine calf serum (Hyclone).

5 Recombinant vectors

[0073] The ERβ-encoding sequence as presented in SEQ ID No. 1 was amplified by PCR using oligonucleotides 5'-CTTGGATCCATAGCCCTGCTGTGATGAATTACAG-3' (SEQ ID NO:22 underlined is the translation initiation codon) in combination with 5'-GATGGATCCTCACCTCAGGGCCAGGCGTCACTG-3' (SEQ ID NO:23) (underlined is the translation stopcodon, antisense). The resulting BamH1 fragment (approximately 1450 base pairs) were then cloned in the mammalian cell expression vector pNGV1 (Genbank accession No. X99274).

10 [0074] An expression construct encoding the ERβ reading frame as presented in SEQ ID NO:24 was made by replacing a BamH1-Msc1 fragment (nucleotides 1-81 in SEQ ID No. 1) by a BamH1-Msc1 fragment corresponding to nucleotides 77-316 in SEQ ID No. 24. The latter fragment was made by PCR with SEQ ID NO:26 in combination with
15 SEQ ID NO:28 using the above mentioned 5' RACE fragment.

[0075] The reporter vector was based on the rat oxytocin gene regulatory region (position -363/+16 as a HindIII/Mbol fragment; R.Ivell, and D.Richter, Proc.Natl.Acad.Sci.USA 81, 2006-2010, 1984) linked to the firefly luciferase encoding sequence; the regulatory region of the oxytocin gene was shown to possess functional estrogen hormone response elements *in vitro* for both the rat (R.Adan *et al*, Biochem.Biophys.Res.Comm. 175, 117-122, 1991) and the
20 human (S.Richard, and H.Zingg, J.Biol.Chem. 265, 6098-6103, 1990).

Transient transfection

[0076] 1 x 10⁵ CHO cells were seeded in 6-wells Nunclon tissue culture plates and DNA was introduced by use of lipofectin (Gibco BRL). Hereto, the DNA (1 µg of both receptor and reporter vector in 250 µL Optimem, Gibco BRL) was mixed with an equal volume of lipofectin reagent (7 µL in 250 µL Optimem, Gibco) and allowed to stand at room temperature for 15 min. After washing the cells twice with serum-free medium (M505) new medium (500 µL Optimem, Gibco) was added to the cells followed by the dropwise addition of the DNA-lipofectin mixture. After incubation for a 5
30 hour period at 37°C cells were washed twice with fenolred-free M505 + 5% charcoal-treated bovine calf serum and incubated overnight at 37°C. After 24 hours hormones were added to the medium (10⁻⁷ mol/L). Cell extracts were made 48 hours posttransfection by the addition of 200 µL lysisbuffer (0.1 M phosphate buffer pH7.8, 0.2% Triton X-100). After incubation for 5 min at 37°C the cell suspension was centrifuged (Eppendorf centrifuge, 5 min) and 20 µL sample was added to 50 µL luciferase assay reagent (Promega). Light emission was measured in a luminometer (Berthold Biolumat) for 10 sec at 562 nm.

Stable transfection of the novel estrogen receptor.

[0077] The expression plasmid encoding full-length ERβ1-530 (see above) was stably transfected in CHO K1 cells as previously described (Theunissen *et al.*, J. Biol. Chem. 268, 9035-9040, 1993). Single cell clones that were obtained
40 this way were screened by transient transfection of the reporter plasmid (rat oxytocin-luciferase) as described above. Selected clones were used for a second stable transfection of the rat oxytocin-luciferase reporter plasmid together with the plasmid pDR2A which contains a hygromycine resistance gene for selection. Single cell clones obtained were tested for a response to 17β-estradiol. Subsequently, a selected single cell clone was used for transactivation studies. Briefly, cells were seeded in 96-wells at (1.6x10⁴ cells per well). After 24 hours different concentrations of hormone
45 were diluted in medium and added to the wells. For antagonistic experiments, 2x10⁻¹⁰ M. 17β-estradiol was added to each well and different concentrations of antagonists were added. Cells were washed once with PBS after a 24 hour incubation and then lysed by the addition of 40 microliter lysis buffer (see above). Luciferase reagent was added (50 microliter) to each well and light emission was measured using the Topcount (Packard).

50 Results.

[0078] A comparison of the two expression constructs (SEQ ID NO:1 and SEQ ID NO:24) in transient transfections in CHO cells showed identical transactivation in response to a number of agonists and antagonists. CHO cells transiently transfected with ERβ expression vector and a reporter plasmid showed a 3 to 4 fold increase in luciferase activity
55 in response to 17β-estradiol as compared to untreated cells (see Figure 2). A similar transactivation was obtained upon treatment with estriol and estrone. The results indicate not only that the novel ER (ERβ) can bind estrogen hormones but also that the ligand-activated receptor can bind to the estrogen-response elements (EREs) within the rat oxytocin promoter and activate transcription of the luciferase reporter gene. Figure 3 shows that in an independent similar

experiment 10^{-9} mol/L 17β -estradiol gave an 18-fold stimulation with $ER\alpha$ and a 7-fold stimulation with $ER\beta$. In addition, the antiestrogen ICI-164384 was shown to be an antagonist for both $ER\alpha$ and $ER\beta$ when activated with 17β -estradiol, whereas the antagonist alone had no effect. In this experiment 0.25 μ g β -galactosidase vector was co-transfected in order to normalize for differences in transfection efficiency.

[0079] Transactivation studies performed on stably transfected $ER\alpha$ and $ER\beta$ cell lines gave similar absolute luciferase values. The curves for 17β -estradiol are very similar and show that half-maximal transactivation is reached with lower concentrations of hormone on $ER\alpha$ as compared to $ER\beta$ (Figure 5). For Org4094 this is also the case however, the effect observed is much more pronounced. The curves for raloxifen show that the potency of this antagonist to block transactivation on $ER\alpha$ is greater compared to its potency to block $ER\beta$ transactivation.

SEQUENCE LISTING

[0080]

(1) GENERAL INFORMATION:

(i) APPLICANT:

- (A) NAME: Akzo nobel n.v.
- (B) STREET: Velperweg 76
- (C) CITY: Arnhem
- (E) COUNTRY: The Netherlands
- (F) POSTAL CODE (ZIP): 6824 BM
- (G) TELEPHONE: 0412-666379
- (H) TELEFAX: 0412-650592
- (I) TELEX: 37503 akpha nl

(ii) TITLE OF INVENTION: Novel estrogen receptor

(iii) NUMBER OF SEQUENCES: 28

(iv) COMPUTER READABLE FORM:

- (A) MEDIUM TYPE: Floppy disk
- (B) COMPUTER: IBM PC compatible
- (C) OPERATING SYSTEM: PC-DOS/MS-DOS
- (D) SOFTWARE: PatentIn Release #1.0, Version #1.30 (EPO)

(2) INFORMATION FOR SEQ ID NO: 1:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 1434 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: double
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 1:

EP 0 798 378 B1

	ATGAATTACA GCATTCCCAG CAATGTCACT AACTTGAAG GTGGGCCTGG TCGGCAGACC	60
5	ACAAGCCCAA ATGTGTTGTG GCCAACACCT GGGCACCTTT CTCCTTTAGT GGTCCATCGC	120
	CAGTTATCAC ATCTGTATGC GGAACCTCAA AAGAGTCCCT GGTGTGAAGC AAGATCGCTA	180
10	GAACACACCT TACCTGTAAA CAGAGAGACA CTGAAAAGGA AGGTTAGTGG GAACCGTTGC	240
	GCCAGCCCTG TTACTGGTCC AGGTTCAAAG AGGGATGCTC ACTTCTGCGC TGTCTGCAGC	300
15	GATTACGCAT CGGGATATCA CTATGGAGTC TGGTCGTGTG AAGGATGTAA GGCCTTTTTT	360
	AAAAGAAGCA TTCAAGGACA TAATGATTAT ATTTGTCCAG CTACAAATCA GTGTACAATC	420
	GATAAAAACC GGCACAAGAG CTGCCAGGCC TGCCGACTTC GGAAGTGTTA CGAAGTGGGA	480
25	ATGGTGAAGT GTGGCTCCCG GAGAGAGAGA TGTGGGTACC GCCTTGTGCG GAGACAGAGA	540
	AGTGCCGACG AGCAGCTGCA CTGTGCCGGC AAGGCCAAGA GAAGTGCGCG CCACGCGCCC	600
30	CGAGTGCGGG AGCTGCTGCT GGACGCCCTG AGCCCCGAGC AGCTAGTGCT CACCCTCCTG	660
	GAGGCTGAGC CGCCCCATGT GCTGATCAGC CGCCCCAGTG CGCCCTTCAC CGAGGCCTCC	720
35	ATGATGATGT CCCTGACCAA GTTGGCCGAC AAGGAGTTGG TACACATGAT CAGCTGGGCC	780
	AAGAAGATTC CCGGCTTTGT GGAGCTCAGC CTGTTCGACC AAGTGCGGCT CTGGAGAGC	840
	TGTTGGATGG AGGTGTTAAT GATGGGGCTG ATGTGGCGCT CAATTGACCA CCCC GGCAAG	900
45	CTCATCTTTG CTCCAGATCT TGTCTGGAC AGGGATGAGG GGAAATGCGT AGAAGGAATT	960
	CTGGAAATCT TTGACATGCT CCTGGCAACT ACTTCAAGGT TTCGAGAGTT AAAACTCCAA	1020
50	CACAAAGAAT ATCTCTGTGT CAAGGCCATG ATCCTGCTCA ATTCCAGTAT GTACCCTCTG	1080
	GTCACAGCGA CCCAGGATGC TGACAGCAGC CGGAAGCTGG CTCACTTGCT GAACGCCGTG	1140
55		

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ACCGATGCTT TGGTTTGGGT GATTGCCAAG AGCGGCATCT CCTCCCAGCA GCAATCCATG 1200
 5 CGCCTGGCTA ACCTCCTGAT GCTCCTGTCC CACGTCAGGC ATGCGAGTAA CAAGGGCATG 1260
 GAACATCTGC TCAACATGAA GTGCAAAAAT GTGGTCCCAG TGTATGACCT GCTGCTGGAG 1320
 10 ATGCTGAATG CCCACGTGCT TCGCGGGTGC AAGTCCTCCA TCACGGGGTC CGAGTGCAGC 1380
 CCGGCAGAGG ACAGTAAAAG CAAAGAGGGC TCCAGAACC CACAGTCTCA GTGA 1434

15 (2) INFORMATION FOR SEQ ID NO: 2:
 (i) SEQUENCE CHARACTERISTICS:
 20 (A) LENGTH: 1251 base pairs
 (B) TYPE: nucleic acid
 (C) STRANDEDNESS: double
 (D) TOPOLOGY: linear
 25 (ii) MOLECULE TYPE: cDNA
 (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 2:

30 ATGAATTACA GCATTCCCAG CAATGTCACT AACTTGAAG GTGGGCCTGG TCGGCAGACC 60
 ACAAGCCCAA ATGTGTTGTG GCCAACACCT GGGCACCTTT CTCCTTTAGT GGTCCATCGC 120
 35 CAGTTATCAC ATCTGTATGC GGAACCTCAA AAGAGTCCCT GGTGTGAAGC AAGATCGCTA 180
 GAACACACCT TACCTGTAAA CAGAGAGACA CTGAAAAGGA AGGTTAGTGG GAACCGTTGC 240
 40 GCCAGCCCTG TTA CTGGTCC AGGTTCAAAG AGGGATGCTC ACTTCTGCGC TGTCTGCAGC 300
 GATTACGCAT CGGGATATCA CTATGGAGTC TGGTCGTGTG AAGGATGTAA GGCCTTTTTT 360
 AAAAGAAGCA TTCAAGGACA TAATGATTAT ATTTGTCCAG CTACAAATCA GTGTACAATC 420
 50 GATAAAAACC GCGCAAGAG CTGCCAGGCC TGCCGACTTC GGAAGTGTTA CGAAGTGGGA 480

55

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5 40
 ATGGTGAAGT GTGGCTCCCG GAGAGAGAGA TGTGGGTACC GCCTTGTGCG GAGACAGAGA 540
 5 AGTGCCGACG AGCAGCTGCA CTGTGCCGGC AAGGCCAAGA GAAGTGGCGG CCACGCGCCC 600
 CGAGTGCGGG AGCTGCTGCT GGACGCCCTG AGCCCCGAGC AGCTAGTGCT CACCCTCCTG 660
 10 GAGGCTGAGC CGCCCCATGT GCTGATCAGC CGCCCCAGTG CGCCCTTCAC CGAGGCCTCC 720
 ATGATGATGT CCCTGACCAA GTTGGCCGAC AAGGAGTTGG TACACATGAT CAGCTGGGCC 780
 15 AAGAAGATTC CCGGCTTTGT GGAGCTCAGC CTGTTCGACC AAGTGCGGCT CTTGGAGAGC 840
 TGTTGGATGG AGGTGTTAAT GATGGGGCTG ATGTGGCGCT CAATTGACCA CCCC GGCAAG 900
 CTCATCTTTG CTCCAGATCT TGTTCTGGAC AGGGATGAGG GGAAATGCGT AGAAGGAATT 960
 20 CTGGAATCT TTGACATGCT CCTGGCAACT ACTTCAAGGT TTCGAGAGTT AAAACTCCAA 1020
 CACAAAGAAT ATCTCTGTGT CAAGGCCATG ATCCTGCTCA ATTCCAGTAT GTACCCTCTG 1080
 30 GTCACAGCGA CCCAGGATGC TGACAGCAGC CGGAAGCTGG CTCACTTGCT GAACGCCGTG 1140
 ACCGATGCTT TGTTTGGGT GATTGCCAAG AGCGGCATCT CCTCCAGCA GCAATCCATG 1200
 35 CGCCTGGCTA ACCTCCTGAT GCTCCTGTCC CACGTCAGGC ATGCGAGGTG A 1251

40 (2) INFORMATION FOR SEQ ID NO: 3:

(i) SEQUENCE CHARACTERISTICS:

- 45 (A) LENGTH: 66 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

50 (ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 3:

55

EP 0 798 378 B1

Cys Ala Val Cys Ser Asp Tyr Ala Ser Gly Tyr His Tyr Gly Val Trp
 1 5 10 15

5

Ser Cys Glu Gly Cys Lys Ala Phe Phe Lys Arg Ser Ile Gln Gly His
 20 25 30

10

Asn Asp Tyr Ile Cys Pro Ala Thr Asn Gln Cys Thr Ile Asp Lys Asn
 35 40 45

15

Arg Arg Lys Ser Cys Gln Ala Cys Arg Leu Arg Lys Cys Tyr Glu Val
 50 55 60

20

Gly Met
 65

(2) INFORMATION FOR SEQ ID NO: 4:

25

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 233 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

30

(ii) MOLECULE TYPE: peptide

35

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 4:

Leu Val Leu Thr Leu Leu Glu Ala Glu Pro Pro His Val Leu Ile Ser
 1 5 10 15

40

Arg Pro Ser Ala Pro Phe Thr Glu Ala Ser Met Met Met Ser Leu Thr
 20 25 30

45

Lys Leu Ala Asp Lys Glu Leu Val His Met Ile Ser Trp Ala Lys Lys
 35 40 45

50

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EP 0 798 378 B1

Ile Pro Gly Phe Val Glu Leu Ser Leu Phe Asp Gln Val Arg Leu Leu
 50 55 60
 5
 Glu Ser Cys Trp Met Glu Val Leu Met Met Gly Leu Met Trp Arg Ser
 65 70 75 80
 10
 Ile Asp His Pro Gly Lys Leu Ile Phe Ala Pro Asp Leu Val Leu Asp
 85 90 95
 15
 Arg Asp Glu Gly Lys Cys Val Glu Gly Ile Leu Glu Ile Phe Asp Met
 100 105 110
 20
 Leu Leu Ala Thr Thr Ser Arg Phe Arg Glu Leu Lys Leu Gln His Lys
 115 120 125
 25
 Glu Tyr Leu Cys Val Lys Ala Met Ile Leu Leu Asn Ser Ser Met Tyr
 130 135 140
 30
 Pro Leu Val Thr Ala Thr Gln Asp Ala Asp Ser Ser Arg Lys Leu Ala
 145 150 155 160
 35
 His Leu Leu Asn Ala Val Thr Asp Ala Leu Val Trp Val Ile Ala Lys
 165 170 175
 40
 Ser Gly Ile Ser Ser Gln Gln Gln Ser Met Arg Leu Ala Asn Leu Leu
 180 185 190
 45
 Met Leu Leu Ser His Val Arg His Ala Ser Asn Lys Gly Met Glu His
 195 200 205
 50
 Leu Leu Asn Met Lys Cys Lys Asn Val Val Pro Val Tyr Asp Leu Leu
 210 215 220
 Leu Glu Met Leu Asn Ala His Val Leu
 225 230

(2) INFORMATION FOR SEQ ID NO: 5:

55 (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 477 amino acids
- (B) TYPE: amino acid

EP 0 798 378 B1

(C) STRANDEDNESS: single

(D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: protein

5

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 5:

10 Met Asn Tyr Ser Ile Pro Ser Asn Val Thr Asn Leu Glu Gly Gly Pro
 1 5 10 15

15 Gly Arg Gln Thr Thr Ser Pro Asn Val Leu Trp Pro Thr Pro Gly His
 20 25 30

20 Leu Ser Pro Leu Val Val His Arg Gln Leu Ser His Leu Tyr Ala Glu
 35 40 45

25 Pro Gln Lys Ser Pro Trp Cys Glu Ala Arg Ser Leu Glu His Thr Leu
 50 55 60

30 Pro Val Asn Arg Glu Thr Leu Lys Arg Lys Val Ser Gly Asn Arg Cys
 65 70 75 80

35 Ala Ser Pro Val Thr Gly Pro Gly Ser Lys Arg Asp Ala His Phe Cys
 85 90 95

40 Ala Val Cys Ser Asp Tyr Ala Ser Gly Tyr His Tyr Gly Val Trp Ser
 100 105 110

45 Cys Glu Gly Cys Lys Ala Phe Phe Lys Arg Ser Ile Gln Gly His Asn
 115 120 125

50 Asp Tyr Ile Cys Pro Ala Thr Asn Gln Cys Thr Ile Asp Lys Asn Arg
 130 135 140

55

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Arg Lys Ser Cys Gln Ala Cys Arg Leu Arg Lys Cys Tyr Glu Val Gly
 145 150 155 160
 5
 Met Val Lys Cys Gly Ser Arg Arg Glu Arg Cys Gly Tyr Arg Leu Val
 165 170 175
 10
 Arg Arg Gln Arg Ser Ala Asp Glu Gln Leu His Cys Ala Gly Lys Ala
 180 185 190
 15
 Lys Arg Ser Gly Gly His Ala Pro Arg Val Arg Glu Leu Leu Leu Asp
 195 200 205
 20
 Ala Leu Ser Pro Glu Gln Leu Val Leu Thr Leu Leu Glu Ala Glu Pro
 210 215 220
 25
 Pro His Val Leu Ile Ser Arg Pro Ser Ala Pro Phe Thr Glu Ala Ser
 225 230 235 240
 30
 Met Met Met Ser Leu Thr Lys Leu Ala Asp Lys Glu Leu Val His Met
 245 250 255
 35
 Ile Ser Trp Ala Lys Lys Ile Pro Gly Phe Val Glu Leu Ser Leu Phe
 260 265 270
 40
 Asp Gln Val Arg Leu Leu Glu Ser Cys Trp Met Glu Val Leu Met Met
 275 280 285
 45
 Gly Leu Met Trp Arg Ser Ile Asp His Pro Gly Lys Leu Ile Phe Ala
 290 295 300
 50
 Pro Asp Leu Val Leu Asp Arg Asp Glu Gly Lys Cys Val Glu Gly Ile
 305 310 315 320
 55
 Leu Glu Ile Phe Asp Met Leu Leu Ala Thr Thr Ser Arg Phe Arg Glu
 325 330 335
 Leu Lys Leu Gln His Lys Glu Tyr Leu Cys Val Lys Ala Met Ile Leu
 340 345 350

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Leu Asn Ser Ser Met Tyr Pro Leu Val Thr Ala Thr Gln Asp Ala Asp
 355 360 365

5

Ser Ser Arg Lys Leu Ala His Leu Leu Asn Ala Val Thr Asp Ala Leu
 370 375 380

10

Val Trp Val Ile Ala Lys Ser Gly Ile Ser Ser Gln Gln Gln Ser Met
 385 390 395 400

15

Arg Leu Ala Asn Leu Leu Met Leu Leu Ser His Val Arg His Ala Ser
 405 410 415

20

Asn Lys Gly Met Glu His Leu Leu Asn Met Lys Cys Lys Asn Val Val
 420 425 430

25

Pro Val Tyr Asp Leu Leu Leu Glu Met Leu Asn Ala His Val Leu Arg
 435 440 445

30

Gly Cys Lys Ser Ser Ile Thr Gly Ser Glu Cys Ser Pro Ala Glu Asp
 450 455 460

35

Ser Lys Ser Lys Glu Gly Ser Gln Asn Pro Gln Ser Gln
 465 470 475

(2) INFORMATION FOR SEQ ID NO: 6:

(i) SEQUENCE CHARACTERISTICS:

40

- (A) LENGTH: 416 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: unknown

45

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 6:

50

Met Asn Tyr Ser Ile Pro Ser Asn Val Thr Asn Leu Glu Gly Gly Pro

55

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1          5          10          15

5          Gly Arg Gln Thr Thr Ser Pro Asn Val Leu Trp Pro Thr Pro Gly His
              20          25          30

10         Leu Ser Pro Leu Val Val His Arg Gln Leu Ser His Leu Tyr Ala Glu
              35          40          45

15         Pro Gln Lys Ser Pro Trp Cys Glu Ala Arg Ser Leu Glu His Thr Leu
              50          55          60

20         Pro Val Asn Arg Glu Thr Leu Lys Arg Lys Val Ser Gly Asn Arg Cys
              65          70          75          80

25         Ala Ser Pro Val Thr Gly Pro Gly Ser Lys Arg Asp Ala His Phe Cys
              85          90          95

30         Ala Val Cys Ser Asp Tyr Ala Ser Gly Tyr His Tyr Gly Val Trp Ser
              100         105         110

35         Cys Glu Gly Cys Lys Ala Phe Phe Lys Arg Ser Ile Gln Gly His Asn
              115         120         125

40         Asp Tyr Ile Cys Pro Ala Thr Asn Gln Cys Thr Ile Asp Lys Asn Arg
              130         135         140

45         Arg Lys Ser Cys Gln Ala Cys Arg Leu Arg Lys Cys Tyr Glu Val Gly
              145         150         155         160

50         Met Val Lys Cys Gly Ser Arg Arg Glu Arg Cys Gly Tyr Arg Leu Val
              165         170         175

55         Arg Arg Gln Arg Ser Ala Asp Glu Gln Leu His Cys Ala Gly Lys Ala
              180         185         190

60         Lys Arg Ser Gly Gly His Ala Pro Arg Val Arg Glu Leu Leu Leu Asp
              195         200         205

65         Ala Leu Ser Pro Glu Gln Leu Val Leu Thr Leu Leu Glu Ala Glu Pro
    
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	210		215		220															
5	Pro	His	Val	Leu	Ile	Ser	Arg	Pro	Ser	Ala	Pro	Phe	Thr	Glu	Ala	Ser				
	225					230					235					240				
10	Met	Met	Met	Ser	Leu	Thr	Lys	Leu	Ala	Asp	Lys	Glu	Leu	Val	His	Met				
					245					250					255					
15	Ile	Ser	Trp	Ala	Lys	Lys	Ile	Pro	Gly	Phe	Val	Glu	Leu	Ser	Leu	Phe				
				260					265						270					
20	Asp	Gln	Val	Arg	Leu	Leu	Glu	Ser	Cys	Trp	Met	Glu	Val	Leu	Met	Met				
			275					280						285						
25	Gly	Leu	Met	Trp	Arg	Ser	Ile	Asp	His	Pro	Gly	Lys	Leu	Ile	Phe	Ala				
		290					295					300								
30	Pro	Asp	Leu	Val	Leu	Asp	Arg	Asp	Glu	Gly	Lys	Cys	Val	Glu	Gly	Ile				
	305					310					315					320				
35	Leu	Glu	Ile	Phe	Asp	Met	Leu	Leu	Ala	Thr	Thr	Ser	Arg	Phe	Arg	Glu				
					325					330					335					
40	Leu	Lys	Leu	Gln	His	Lys	Glu	Tyr	Leu	Cys	Val	Lys	Ala	Met	Ile	Leu				
				340					345					350						
45	Leu	Asn	Ser	Ser	Met	Tyr	Pro	Leu	Val	Thr	Ala	Thr	Gln	Asp	Ala	Asp				
			355					360						365						
50	Ser	Ser	Arg	Lys	Leu	Ala	His	Leu	Leu	Asn	Ala	Val	Thr	Asp	Ala	Leu				
		370					375						380							
55	Val	Trp	Val	Ile	Ala	Lys	Ser	Gly	Ile	Ser	Ser	Gln	Gln	Gln	Ser	Met				
	385					390					395					400				
60	Arg	Leu	Ala	Asn	Leu	Leu	Met	Leu	Leu	Ser	His	Val	Arg	His	Ala	Arg				
				405						410					415					

(2) INFORMATION FOR SEQ ID NO: 7:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 29 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: both
- (D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 7:

GGIGAYGARG CWTICGGITG YCAYTAYGG

29

(2) INFORMATION FOR SEQ ID NO: 8:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 29 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 8:

AAGCCTGSSA YICKYTTIGC CCAIYTIAT

29

(2) INFORMATION FOR SEQ ID NO: 9:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 22 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 9:

TGTTACGAAG TGGGAATGGT GA

22

(2) INFORMATION FOR SEQ ID NO: 10:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 24 base pairs
- (B) TYPE: nucleic acid

- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

5

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 10:

TTGACACCAG ACCAACTGGT AATG

10

24

(2) INFORMATION FOR SEQ ID NO: 11:

(i) SEQUENCE CHARACTERISTICS:

15

- (A) LENGTH: 24 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single .
- (D) TOPOLOGY: linear

20

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 11:

25

GGTGGCGACG ACTCCTGGAG CCCG

24

(2) INFORMATION FOR SEQ ID NO: 12:

30

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 22 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

35

(ii) MOLECULE TYPE: cDNA

40

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 12:

GTACACTGAT TTGTAGCTGG AC

22

45

(2) INFORMATION FOR SEQ ID NO: 13:

(i) SEQUENCE CHARACTERISTICS:

50

- (A) LENGTH: 20 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

55

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 13:

CCATGATGAT GTCCCTGACC

20

5 (2) INFORMATION FOR SEQ ID NO: 14:

(i) SEQUENCE CHARACTERISTICS:

- 10 (A) LENGTH: 20 base pairs
 (B) TYPE: nucleic acid
 (C) STRANDEDNESS: single
 (D) TOPOLOGY: linear

15 (ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 14:

20 TCGCATGCCT GACGTGGGAC

20

(2) INFORMATION FOR SEQ ID NO: 15:

(i) SEQUENCE CHARACTERISTICS:

- 25 (A) LENGTH: 24 base pairs
 (B) TYPE: nucleic acid
 (C) STRANDEDNESS: single
 (D) TOPOLOGY: linear

30 (ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 15:

35 GGCSTCCAGC ATCTCCAGSA RCAG

24

40 (2) INFORMATION FOR SEQ ID NO: 16:

(i) SEQUENCE CHARACTERISTICS:

- 45 (A) LENGTH: 20 base pairs
 (B) TYPE: nucleic acid
 (C) STRANDEDNESS: single
 (D) TOPOLOGY: linear

50 (ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 16:

55 GGAAGCTGGC TCACTTGCTG

20

(2) INFORMATION FOR SEQ ID NO: 17:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 20 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 17:

TCTTGGTTCTG GACAGGGATG

20

(2) INFORMATION FOR SEQ ID NO: 18:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 20 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 18:

GCATGGAACA TCTGCTCAAC

20

(2) INFORMATION FOR SEQ ID NO: 19:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 21 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 19:

AGCAAGTTCA GCCTGTTAAG T

21

(2) INFORMATION FOR SEQ ID NO: 20:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 1257 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: double
- (D) TOPOLOGY: linear

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(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 20:

5
ATGAATTACA GCATTCCCAG CAATGTCACT AACTTGAAG GTGGGCCTGG TCGGCAGACC 60
10
ACAAGCCCAA ATGTGTTGTG GCCAACACCT GGGCACCTTT CTCCTTTAGT GGTCCATCGC 120
CAGTTATCAC ATCTGTATGC GGAACCTCAA AAGAGTCCCT GGTGTGAAGC AAGATCGCTA 180
15
GAACACACCT TACCTGTAAA CAGAGAGACA CTGAAAAGGA AGGTTAGTGG GAACCGTTGC 240
GCCAGCCCTG TTAGTGTCC AGGTTCAAAG AGGGATGCTC ACTTCTGCGC TGTCTGCAGC 300
20
GATTACGCAT CGGGATATCA CTATGGAGTC TGGTCGTGTG AAGGATGTAA GGCCTTTTTT 360

25

30

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	AAAAGAAGCA TTCAAGGACA TAATGATTAT ATTTGTCCAG CTACAAATCA GTGTACAATC	420
5	GATAAAAACC GGCACAAGAG CTGCCAGGCC TGCCGACTTC GGAAGTGTTA CGAAGTGGGA	480
	ATGGTGAAGT GTGGCTCCCG GAGAGAGAGA TGTGGGTACC GCCTTGTGCG GAGACAGAGA	540
10	AGTGCCGACG AGCAGCTGCA CTGTGCCGGC AAGGCCAAGA GAAGTGGCGG CCACGCGCCC	600
	CGAGTGCGGG AGCTGCTGCT GGACGCCCTG AGCCCCGAGC AGCTAGTGCT CACCCTCCTG	660
15	GAGGCTGAGC CGCCCCATGT GCTGATCAGC CGCCCCAGTG CGCCCTTAC CGAGGCCTCC	720
	ATGATGATGT CCCTGACCAA GTTGCCGAC AAGGAGTTGG TACACATGAT CAGCTGGGCC	780
20	AAGAAGATTC CCGGCTTTGT GGAGCTCAGC CTGTTGACC AAGTGCGGCT CTTGGAGAGC	840
	TGTTGGATGG AGGTGTTAAT GATGGGGCTG ATGTGGCGCT CAATTGACCA CCCCAGCAAG	900
25	CTCATCTTTG CTCCAGATCT TGTCTGGAC AGGGATGAGG GGAAATGCGT AGAAGGAATT	960
	CTGGAAATCT TTGACATGCT CCTGGCAACT ACTTCAAGGT TTCGAGAGTT AAAACTCCAA	1020
30	CACAAAGAAT ATCTCTGTGT CAAGGCCATG ATCCTGCTCA ATTCCAGTAT GTACCCTCTG	1080
35	GTCACAGCGA CCCAGGATGC TGACAGCAGC CGGAAGCTGG CTCACTTGCT GAACGCCGTG	1140
	ACCGATGCTT TGTTTTGGGT GATTGCCAAG AGCGGCATCT CCTCCAGCA GCAATCCATG	1200
40	CGCCTGGCTA ACCTCCTGAT GTCCTGTCC CACGTCAGGC ATGCGAGGTC TGCCTGA	1257

45 (2) INFORMATION FOR SEQ ID NO: 21:

(i) SEQUENCE CHARACTERISTICS:

50 (A) LENGTH: 418 amino acids
 (B) TYPE: amino acid
 (C) STRANDEDNESS: single
 (D) TOPOLOGY: linear

55 (ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 21:

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1 Met Asn Tyr Ser Ile Pro Ser Asn Val Thr Asn Leu Glu Gly Gly Pro
 5 5 10 15
 Gly Arg Gln Thr Thr Ser Pro Asn Val Leu Trp Pro Thr Pro Gly His
 10 20 25 30
 Leu Ser Pro Leu Val Val His Arg Gln Leu Ser His Leu Tyr Ala Glu
 15 35 40 45
 Pro Gln Lys Ser Pro Trp Cys Glu Ala Arg Ser Leu Glu His Thr Leu
 20 50 55 60
 Pro Val Asn Arg Glu Thr Leu Lys Arg Lys Val Ser Gly Asn Arg Cys
 25 65 70 75 80
 Ala Ser Pro Val Thr Gly Pro Gly Ser Lys Arg Asp Ala His Phe Cys
 30 85 90 95
 Ala Val Cys Ser Asp Tyr Ala Ser Gly Tyr His Tyr Gly Val Trp Ser
 35 100 105 110
 Cys Glu Gly Cys Lys Ala Phe Phe Lys Arg Ser Ile Gln Gly His Asn
 40 115 120 125
 Asp Tyr Ile Cys Pro Ala Thr Asn Gln Cys Thr Ile Asp Lys Asn Arg
 45 130 135 140
 Arg Lys Ser Cys Gln Ala Cys Arg Leu Arg Lys Cys Tyr Glu Val Gly
 50 145 150 155 160
 Met Val Lys Cys Gly Ser Arg Arg Glu Arg Cys Gly Tyr Arg Leu Val
 55 165 170 175
 Arg Arg Gln Arg Ser Ala Asp Glu Gln Leu His Cys Ala Gly Lys Ala

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		180						185					190			
5	Lys	Arg	Ser	Gly	Gly	His	Ala	Pro	Arg	Val	Arg	Glu	Leu	Leu	Leu	Asp
			195					200					205			
10	Ala	Leu	Ser	Pro	Glu	Gln	Leu	Val	Leu	Thr	Leu	Leu	Glu	Ala	Glu	Pro
		210					215					220				
15	Pro	His	Val	Leu	Ile	Ser	Arg	Pro	Ser	Ala	Pro	Phe	Thr	Glu	Ala	Ser
	225					230					235					240
20	Met	Met	Met	Ser	Leu	Thr	Lys	Leu	Ala	Asp	Lys	Glu	Leu	Val	His	Met
				245						250					255	
25	Ile	Ser	Trp	Ala	Lys	Lys	Ile	Pro	Gly	Phe	Val	Glu	Leu	Ser	Leu	Phe
			260						265						270	
30	Asp	Gln	Val	Arg	Leu	Leu	Glu	Ser	Cys	Trp	Met	Glu	Val	Leu	Met	Met
		275						280					285			
35	Gly	Leu	Met	Trp	Arg	Ser	Ile	Asp	His	Pro	Gly	Lys	Leu	Ile	Phe	Ala
	290						295					300				
40	Pro	Asp	Leu	Val	Leu	Asp	Arg	Asp	Glu	Gly	Lys	Cys	Val	Glu	Gly	Ile
	305					310					315					320
45	Leu	Glu	Ile	Phe	Asp	Met	Leu	Leu	Ala	Thr	Thr	Ser	Arg	Phe	Arg	Glu
				325						330					335	
50	Leu	Lys	Leu	Gln	His	Lys	Glu	Tyr	Leu	Cys	Val	Lys	Ala	Met	Ile	Leu
			340						345					350		
55	Leu	Asn	Ser	Ser	Met	Tyr	Pro	Leu	Val	Thr	Ala	Thr	Gln	Asp	Ala	Asp
		355						360					365			
60	Ser	Ser	Arg	Lys	Leu	Ala	His	Leu	Leu	Asn	Ala	Val	Thr	Asp	Ala	Leu
	370						375					380				
65	Val	Trp	Val	Ile	Ala	Lys	Ser	Gly	Ile	Ser	Ser	Gln	Gln	Gln	Ser	Met

385 390 395 400

5 Arg Leu Ala Asn Leu Leu Met Leu Leu Ser His Val Arg His Ala Arg
405 410 415

10 Ser Ala

(2) INFORMATION FOR SEQ ID NO: 22:

15 (i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 34 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
20 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 22:

25

CTTGGATCCA TAGCCCTGCT GTGATGAATT ACAG 34

30 (2) INFORMATION FOR SEQ ID NO: 23:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 33 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
35 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

40

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 23:

45 **GATGGATCCT CACCTCAGGG CCAGGCGTCA CTG 33**

(2) INFORMATION FOR SEQ ID NO: 24:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 1898 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
50 (D) TOPOLOGY: linear

55

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 24:

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	CACGAATCTT TGAGAACATT ATAATGACCT TTGTGCCTCT TCTTGCAAGG TGTTTTCTCA	60
5	GCTGTTATCT CAAGACATGG ATATAAAAAA CTCACCATCT AGCCTTAATT CTCCTTCCTC	120
	CTACAACTGC AGTCAATCCA TCTTACCCCT GGAGCACGGC TCCATATACA TACCTTCCTC	180
10	CTATGTAGAC AGCCACCATG AATATCCAGC CATGACATTC TATAGCCCTG CTGTGATGAA	240
	TTACAGCATT CCCAGCAATG TACTAACTT GGAAGGTGGG CCTGGTCGGC AGACCACAAG	300
15	CCCAAATGTG TTGTGGCCAA CACCTGGGCA CCTTTCTCCT TTAGTGGTCC ATCGCCAGTT	360
	ATCACATCTG TATGCGGAAC CTCAAAAGAG TCCCTGGTGT GAAGCAAGAT CGCTAGAACA	420
20	CACCTTACCT GTAAACAGAG AGACACTGAA AAGGAAGGTT AGTGGGAACC GTTGCGCCAG	480
	CCCTGTTACT GGTCCAGGTT CAAAGAGGGA TGCTCACTTC TGCCTGTCT GCAGCGATTA	540
25	CGCATCGGGA TATCACTATG GAGTCTGGTC GTGTGAAGGA TGTAAGGCCT TTTTAAAAG	600
	AAGCATTCAA GGACATAATG ATTATATTTG TCCAGCTACA AATCAGTGTA CAATCGATAA	660
30	AAACCGGCGC AAGAGCTGCC AGGCCTGCCG ACTTCGGAAG TGTTACGAAG TGGGAATGGT	720
35		
40		
45		
50		
55		

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	GAAGTGTGGC TCCCGGAGAG AGAGATGTGG GTACCGCCTT GTGCGGAGAC AGAGAAGTGC	780
5	CGACGAGCAG CTGCACTGTG CCGGCAAGGC CAAGAGAAGT GCGCGCCACG CGCCCCGAGT	840
	GCGGGAGCTG CTGCTGGACG CCCTGAGCCC CGAGCAGCTA GTGCTCACCC TCCTGGAGGC	900
10	TGAGCCGCCC CATGTGCTGA TCAGCCGCCC CAGTGCGCC TTCACCGAGG CCTCCATGAT	960
	GATGTCCCTG ACCAAGTTGG CCGACAAGGA GTTGGTACAC ATGATCAGCT GGGCCAAGAA	1020
15	GATTCCCGGC TTTGTGGAGC TCAGCCTGTT CGACCAAGTG CGGCTCTTGG AGAGCTGTTG	1080
	GATGGAGGTG TTAATGATGG GGCTGATGTG GCGCTCAATT GACCACCCCG GCAAGCTCAT	1140
20	CTTTGCTCCA GATCTTGTTT TGGACAGGGA TGAGGGGAAA TGCGTAGAAG GAATTCTGGA	1200
	AATCTTTGAC ATGCTCCTGG CAACTACTTC AAGGTTTCGA GAGTTAAAAC TCCAACACAA	1260
25	AGAATATCTC TGTGTCAAGG CCATGATCCT GCTCAATTCC AGTATGTACC CTCTGGTCAC	1320
	AGCGACCCAG GATGCTGACA GCAGCCGGAA GCTGGCTCAC TTGCTGAACG CCGTGACCGA	1380
30	TGCTTTGGTT TGGGTGATTG CCAAGAGCGG CATCTCCTCC CAGCAGCAAT CCATGCGCCT	1440
	GGCTAACCTC CTGATGCTCC TGTCCCACGT CAGGCATGCG AGTAACAAGG GCATGGAACA	1500
35	TCTGCTCAAC ATGAAGTGCA AAAATGTGGT CCCAGTGTAT GACCTGCTGC TGGAGATGCT	1560
	GAATGCCAC GTGCTTCGCG GGTGCAAGTC CTCCATCACG GGGTCCGAGT GCAGCCCGGC	1620
40	AGAGGACAGT AAAAGCAAAG AGGGCTCCCA GAACCCACAG TCTCAGTGAC GCCTGGCCCT	1680
	GAGGTGAACT GGCCACAGA GGTCAACAAGC TGAAGCGTGA ACTCCAGTGT GTCAGGAGCC	1740
45	TGGGCTTCAT CTTTCTGCTG TGTGGTCCCT CATTTGGTGA TGGCAGGCTT GGTCATGTAC	1800
	CATCCTTCCC TCCACCTTCC CAACTCTCAG GAGTCGGTGT GAGGAAGCCA TAGTTTCCCT	1860
50		
55		

TGTTAGCAGA GGGACATTTG AATCGAGCGT TTCCACAC

1898

5

(2) INFORMATION FOR SEQ ID NO: 25:

(i) SEQUENCE CHARACTERISTICS:

- 10 (A) LENGTH: 530 amino acids
 (B) TYPE: amino acid
 (C) STRANDEDNESS: single
 (D) TOPOLOGY: linear

15 (ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 25:

20 Met Asp Ile Lys Asn Ser Pro Ser Ser Leu Asn Ser Pro Ser Ser Tyr
 1 5 10 15

25 Asn Cys Ser Gln Ser Ile Leu Pro Leu Glu His Gly Ser Ile Tyr Ile
 20 25 30

30 Pro Ser Ser Tyr Val Asp Ser His His Glu Tyr Pro Ala Met Thr Phe
 35 40 45

35 Tyr Ser Pro Ala Val Met Asn Tyr Ser Ile Pro Ser Asn Val Thr Asn
 50 55 60

40 Leu Glu Gly Gly Pro Gly Arg Gln Thr Thr Ser Pro Asn Val Leu Trp
 65 70 75 80

45 Pro Thr Pro Gly His Leu Ser Pro Leu Val Val His Arg Gln Leu Ser
 85 90 95

50 His Leu Tyr Ala Glu Pro Gln Lys Ser Pro Trp Cys Glu Ala Arg Ser
 100 105 110

55 Leu Glu His Thr Leu Pro Val Asn Arg Glu Thr Leu Lys Arg Lys Val

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	115		120		125																													
5	Ser	Gly	Asn	Arg	Cys	Ala	Ser	Pro	Val	Thr	Gly	Pro	Gly	Ser	Lys	Arg																		
	130						135					140																						
10	Asp	Ala	His	Phe	Cys	Ala	Val	Cys	Ser	Asp	Tyr	Ala	Ser	Gly	Tyr	His																		
	145					150					155					160																		
15	Tyr	Gly	Val	Trp	Ser	Cys	Glu	Gly	Cys	Lys	Ala	Phe	Phe	Lys	Arg	Ser																		
					165					170						175																		
20	Ile	Gln	Gly	His	Asn	Asp	Tyr	Ile	Cys	Pro	Ala	Thr	Asn	Gln	Cys	Thr																		
			180						185						190																			
25	Ile	Asp	Lys	Asn	Arg	Arg	Lys	Ser	Cys	Gln	Ala	Cys	Arg	Leu	Arg	Lys																		
		195						200							205																			
30	Cys	Tyr	Glu	Val	Gly	Met	Val	Lys	Cys	Gly	Ser	Arg	Arg	Glu	Arg	Cys																		
		210						215							220																			
35	Gly	Tyr	Arg	Leu	Val	Arg	Arg	Gln	Arg	Ser	Ala	Asp	Glu	Gln	Leu	His																		
		225					230						235			240																		
40	Cys	Ala	Gly	Lys	Ala	Lys	Arg	Ser	Gly	Gly	His	Ala	Pro	Arg	Val	Arg																		
					245							250				255																		
45	Glu	Leu	Leu	Leu	Asp	Ala	Leu	Ser	Pro	Glu	Gln	Leu	Val	Leu	Thr	Leu																		
				260						265						270																		
50	Leu	Glu	Ala	Glu	Pro	Pro	His	Val	Leu	Ile	Ser	Arg	Pro	Ser	Ala	Pro																		
				275					280							285																		
55	Phe	Thr	Glu	Ala	Ser	Met	Met	Met	Ser	Leu	Thr	Lys	Leu	Ala	Asp	Lys																		
		290							295							300																		
60	Glu	Leu	Val	His	Met	Ile	Ser	Trp	Ala	Lys	Lys	Ile	Pro	Gly	Phe	Val																		
		305							310						315		320																	
65	Glu	Leu	Ser	Leu	Phe	Asp	Gln	Val	Arg	Leu	Leu	Glu	Ser	Cys	Trp	Met																		

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			325						330			335				
5	Glu	Val	Leu	Met	Met	Gly	Leu	Met	Trp	Arg	Ser	Ile	Asp	His	Pro	Gly
			340						345				350			
10	Lys	Leu	Ile	Phe	Ala	Pro	Asp	Leu	Val	Leu	Asp	Arg	Asp	Glu	Gly	Lys
			355					360					365			
15	Cys	Val	Glu	Gly	Ile	Leu	Glu	Ile	Phe	Asp	Met	Leu	Leu	Ala	Thr	Thr
			370				375					380				
20	Ser	Arg	Phe	Arg	Glu	Leu	Lys	Leu	Gln	His	Lys	Glu	Tyr	Leu	Cys	Val
	385					390					395					400
25	Lys	Ala	Met	Ile	Leu	Leu	Asn	Ser	Ser	Met	Tyr	Pro	Leu	Val	Thr	Ala
					405					410					415	
30	Thr	Gln	Asp	Ala	Asp	Ser	Ser	Arg	Lys	Leu	Ala	His	Leu	Leu	Asn	Ala
				420					425					430		
35	Val	Thr	Asp	Ala	Leu	Val	Trp	Val	Ile	Ala	Lys	Ser	Gly	Ile	Ser	Ser
			435					440					445			
40	Gln	Gln	Gln	Ser	Met	Arg	Leu	Ala	Asn	Leu	Leu	Met	Leu	Leu	Ser	His
		450					455					460				
45	Val	Arg	His	Ala	Ser	Asn	Lys	Gly	Met	Glu	His	Leu	Leu	Asn	Met	Lys
	465					470					475					480
50	Cys	Lys	Asn	Val	Val	Pro	Val	Tyr	Asp	Leu	Leu	Leu	Glu	Met	Leu	Asn
				485						490					495	
55	Ala	His	Val	Leu	Arg	Gly	Cys	Lys	Ser	Ser	Ile	Thr	Gly	Ser	Glu	Cys
				500					505						510	
60	Ser	Pro	Ala	Glu	Asp	Ser	Lys	Ser	Lys	Glu	Gly	Ser	Gln	Asn	Pro	Gln
			515					520					525			
	Ser	Gln														

530

(2) INFORMATION FOR SEQ ID NO: 26:

(i) SEQUENCE CHARACTERISTICS:

- 5 (A) LENGTH: 30 base pairs
 (B) TYPE: nucleic acid
 (C) STRANDEDNESS: single
 (D) TOPOLOGY: linear

10 (ii) MOLECULE TYPE: other nucleic acid

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 26:

15 **GTGCGGATCC TCTCAAGACA TGGATATAAA** 30

(2) INFORMATION FOR SEQ ID NO: 27:

20 (i) SEQUENCE CHARACTERISTICS:

- 25 (A) LENGTH: 25 base pairs
 (B) TYPE: nucleic acid
 (C) STRANDEDNESS: single
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: other nucleic acid

30 (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 27:

35 **AGTAACAGGG CTGGCGCAAC GGTTT** 25

(2) INFORMATION FOR SEQ ID NO: 28:

40 (i) SEQUENCE CHARACTERISTICS:

- 45 (A) LENGTH: 22 base pairs
 (B) TYPE: nucleic acid
 (C) STRANDEDNESS: single
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: other nucleic acid

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 28:

50 **ACTGGCGATG GACCACTAAA GG** 22

55 **Claims**

1. Isolated estrogen receptor having an N-terminal domain, a DNA-binding domain, and a ligand-binding domain, wherein the amino acid sequence of said DNA-binding domain exhibits at least 80% homology with the amino acid

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sequence shown in SEQ ID NO:3 and the amino acid sequence of said ligand-binding domain of said estrogen receptor exhibits at least 70 % homology with the amino acid sequence shown in SEQ ID NO:4, provided that the estrogen receptor does not have the amino acid sequence:

5 MTFYS PAVMN YSVPG STSNL DGGPV RLSTS PNVLW PTSGH LSPLA
THCQS SLLYA EPQKS PWCEA RSLEH TLPVN RETLK RKLSG SSCAS
PVTSP NAKRD AHFCP VCSDY ASGYH YGVWS CEGCK AFFKR SIQGH
10 NDYIC PATNQ CTIDK NRRKS CQACR LRKCY EVGMV KCGSR RERCG
YRIVR RQRSS SEQVH CLSKA KRNGG HAPRV KELLL STLSP EQLVL
TLLEA EPPNV LVSRP SMPFT EASMM MSLTK LADKE LVHMI GWAKK
15 IPGFV ELSLL DQVRL LESCW MEVLM VGLMW RSIDH PGKLI FAPDL
VLDRD EGKCV EGILE IFDML LATT S RFREL KLQHK EYLCV KAMIL
LNSSM YPLAS ANQEA ESSRK LTHLL NAVTD ALVWV IAKSG ISSQQ
20 QSVRL ANLLM LLSHV RHISN KGMEH LLSMK CKNVV PVYDL LLEML
NAHTL RGYKS SISGS ECSST EDSKN KESSQ NLQSQ

- 25 2. Isolated estrogen receptor according to claim 1, **characterised in that** the amino acid sequence of said DNA-binding domain exhibits at least 90% homology with the amino acid sequence shown in SEQ ID NO: 3.
3. Isolated estrogen receptor according to anyone of claims 1-2, **characterised in that** the amino acid sequence of said ligand-binding domain exhibits at least 75% homology with the amino acid sequence shown in SEQ ID NO: 4.
- 30 4. Isolated estrogen receptor according to anyone of claims 1-3, **characterised in that** said estrogen receptor comprises the amino acid sequence of SEQ ID NO: 5, SEQ ID NO: 6, SEQ ID NO: 21 or SEQ ID NO: 25.
5. Isolated DNA encoding an estrogen receptor according to claims 1-4.
- 35 6. Isolated DNA according to claim 5, **characterised in that** said DNA comprises the nucleic acid sequence of SEQ ID NO: 1, SEQ ID NO:2, SEQ ID NO:20 or SEQ ID NO:24.
7. A recombinant expression vector comprising the DNA according to claim 5 or 6.
- 40 8. A cell transfected with DNA according to claim 5 or 6 or an expression vector according to claim 7.
9. A cell according to claim 8 which is a stable transfected cell line which expresses the estrogen receptor according to any of the claims 1-4.
- 45 10. Use of a DNA according to claim 5 or 6, an expression vector according to claim 7, a cell according to claim 8 or 9, a receptor according to any one of claims 1-4, in a screening assay for identification of new drugs.
- 50 11. Method of identifying functional ligands for a receptor according to any one of claims 1-4, said method comprising the steps of
 - a) introducing into a suitable host cell 1) DNA according to claims 5 or 6, and 2) a suitable reporter gene functionally linked to an operative hormone responsive element (HRE), said HRE being able to be activated by the DNA-binding domain of the receptor encoded by said DNA;
 - b) bringing said host cell from a) into contact with potential ligands which will possibly bind to the ligand-binding domain of the receptor encoded by said DNA from step a); and
 - 55 c) monitoring the expression of the receptor encoded by said reporter gene of step a).

Patentansprüche

1. Isolierter Östrogenrezeptor mit einer N-terminalen Domäne, eine DNA-Bindungsdomäne und einer Ligandenbindungsdomäne, worin die Aminosäuresequenz der genannten DNA-Bindungsdomäne mindestens 80% Homologie mit der in SEQ ID Nr.:3 gezeigten Aminosäuresequenz besitzt und die Aminosäuresequenz der genannten Ligandenbindungsdomäne des genannten Östrogenrezeptors mindestens 70% Homologie mit der in SEQ ID Nr.:4 gezeigten Aminosäuresequenz aufweist, vorausgesetzt, dass der Östrogenrezeptor nicht die folgenden Aminosäuresequenz besitzt:

```

10      MTFYS PAVMN YSVPG STSNL DGGPV RLSTS PNVLW PTSGH LSPLA
      THCQS SLLYA EPQKS PWCEA RSLEH TLPVN RETLK RKLSG SSCAS
      PVTSP NAKRD AHFCP VCS DY ASGYH YGVWS CEGCK AFFKR SIQGH
15      NDYIC PATNQ CTIDK NRRKS CQACR LRKCY EVGMV KCGSR RERCG
      YRIVR RQRSS SEQVH CLSKA KRNGG HAPRV KELLL STLSP EQLVL
      TLLEA EPPNV LVS RP SMPFT EASMM MSLTK LADKE LVHMI GWAKK
20      IPGFV ELSLL DQVRL LESCW MEVLM VGLMW RSIDH PGKLI FAPDL
      VLDRD EGKCV EGILE IFDML LATT S REREL KLQHK EYLCV KAMIL
      LNSSM YPLAS ANQEA ESSRK LTHLL NAVTD ALVWV IAKSG ISSQO
25      QSVRL ANLLM LLSHV RHISN KGMEH LLSMK CKNVV PVYDL LLEML
      NAHTL RGYKS SISGS ECSST EDSKN KESSQ NLQSQ

```

2. Isolierter Östrogenrezeptor nach Anspruch 1, **dadurch gekennzeichnet, dass** die Aminosäuresequenz der genannten DNA-Bindungsdomäne mindestens 90% Homologie mit der in der SEQ ID. Nr.:3 gezeigten Aminosäuresequenz hat.
3. Isolierter Östrogenrezeptor nach einem der Ansprüche 1-2, **dadurch gekennzeichnet, dass** die Aminosäuresequenz der genannten Ligandenbindungsdomäne mindestens 75% Homologie mit der in der SEQ ID. Nr.:4 gezeigten Aminosäuresequenz hat.
4. Isolierter Östrogenrezeptor nach einem der Ansprüche 1-3, **dadurch gekennzeichnet, dass** der genannte Östrogenrezeptor die Aminosäuresequenz der SEQ ID Nr.:5, SEQ ID Nr.:6, SEQ ID Nr.:21 oder SEQ ID Nr.:25 umfasst.
5. Isolierte DNA, welche für einen Östrogenrezeptor nach den Ansprüchen 1-4 codiert.
6. Isolierte DNA nach Anspruch 5, **dadurch gekennzeichnet, dass** die genannte DNA die Nukleinsäuresequenz der SEQ ID Nr.:1, SEQ ID Nr.:2, SEQ ID Nr.:20 oder SEQ ID Nr.:24 umfasst.
7. Rekombinanter Expressionsvektor, welcher die DNA nach einem der Ansprüche 5 oder 6 umfasst.
8. Zelle, welche mit der DNA nach einem der Ansprüche 5 oder 6 oder einem Expressionsvektor nach Anspruch 7 transfiziert ist.
9. Zelle nach Anspruch 8, welche eine stabil transfizierte Zelllinie ist, die den Östrogenrezeptor nach einem der Ansprüche 1-4 exprimiert.
10. Verwendung einer DNA nach einem der Ansprüche 5 oder 6, eines Expressionsvektors nach Anspruch 7, einer Zelle nach Anspruch 8 oder 9, einem Rezeptor nach einem der Ansprüche 1-4 in einem Screening-Assay zur Identifizierung neuer Arzneimittel.
11. Verfahren zur Identifizierung funktionaler Liganden für einen Rezeptor nach einem der Ansprüche 1-4, worin das genannte Verfahren die folgenden Schritte umfasst:

a) Einbringen in eine geeignete Wirtszelle von 1) DNA nach den Ansprüchen 5 oder 6 und 2) von einem geeigneten Reporter-Gen, das funktional mit einem operativen responsiven Hormonelement (HRE) verbunden ist, wobei das genannte HRE fähig ist, von der DNA-Bindungsdomäne des von der genannten DNA codierten Rezeptors aktiviert zu werden;

b) in Kontakt Bringen der genannten Wirtszelle von a) mit potentiellen Liganden, die möglicherweise an die Ligandenbindungsdomäne des von der genannten DNA codierten Rezeptors von Schritt a) binden und
c) Überwachen der Expression des Rezeptors, der von dem genannten Reporter-gen von Schritt a) codiert wird.

Revendications

1. Récepteur d'oestrogène isolé ayant un domaine N-terminal, un domaine de liaison d'ADN, et un domaine de liaison de ligand, dans lequel la séquence d'acides aminés dudit domaine de liaison d'ADN présente au moins 80 % d'homologie avec la séquence d'acides aminés indiquée dans SEQ ID N°3 et la séquence d'acides aminés dudit domaine de liaison de ligand dudit récepteur d'oestrogène présente au moins 70 % d'homologie avec la séquence d'acides aminés indiquée dans SEQ ID N°4, à condition que le récepteur d'oestrogène ne possède pas la séquence d'acides aminés:

```

MTFYS PAVMN YSVPG STSNL DGGPV RLSTS PNVLW PTSGH I.SPLA
THCQS SLLYA EPQKS PWCEA RSLEH TLPVN RETLK RKLSG SSCAS
PVTSP NAKRD AHFCP VCSYD ASGYH YGVWS CEGCK AFFKR SIQGH
NDYIC PA'INQ CT'IDK NRRKS CQACR LRKCY EVGMV KCGSR RERCG
YRIVR RQRSS SEQVH CI.SKA KRNGG HAPRV KELLL STLSP EQLVL
TLLEA EPPNV LVSRP SMPFT EASMM MSLTK LADKE LVHMI GWAKK
IPGFV ELSLL DQVRL LESCW MEVLM VGLMW RSDH PGKLI FAPDL
VLDRD EGKCV EGILE IFDML LATTI RFREL KI.QHK EYLCV KAMII.
LNSSM YPLAS ANQEA ESSRK LTHIL NAVTD ALVWV IAKSG ISSQQ
QSVRL ANLIM LLSHV RHISN KGMEH LLSMK CKNVV PVYDL I.LEML
NAHTL RGYKS SISGS ECSST EDSKN KESSQ NLQSQ

```

2. Récepteur d'oestrogène isolé selon la revendication 1, **caractérisé en ce que** la séquence d'acides aminés dudit domaine de liaison d'ADN présente au moins 90 % d'homologie avec la séquence d'acides aminés indiquée dans SEQ ID N°3.
3. Récepteur d'oestrogène isolé selon l'une quelconque des revendications 1 et 2, **caractérisé en ce que** la séquence d'acides aminés dudit domaine de liaison de ligand présente au moins 75 % d'homologie avec la séquence d'acides aminés indiquée dans SEQ ID N°4.
4. Récepteur d'oestrogène isolé selon l'une quelconque des revendications 1 à 3, **caractérisé en ce que** ledit récepteur d'oestrogène comprend une séquence d'acides aminés de SEQ ID N°5, SEQ ID N°6, SEQ ID N°21 ou SEQ ID N°25.
5. ADN isolé codant pour un récepteur d'oestrogène selon l'une quelconque des revendications 1 à 4.
6. ADN isolé selon la revendication 5, **caractérisé en ce que** ledit ADN comprend la séquence d'acide nucléique de SEQ ID N°1, SEQ ID N°2, SEQ ID N°20 ou SEQ ID N°24.
7. Vecteur d'expression recombinant comprenant l'ADN selon l'une quelconque des revendications 5 ou 6.
8. Cellule transfectée avec l'ADN selon l'une quelconque des revendications 5 ou 6 ou un vecteur d'expression selon la revendication 7.

9. Cellule selon la revendication 8 qui est une lignée cellulaire transfectée stable qui exprime le récepteur d'oestrogène selon l'une quelconque des revendications 1 à 4.

5 10. Utilisation d'un ADN selon l'une quelconque des revendications 5 ou 6, d'un vecteur d'expression selon la revendication 7, d'une cellule selon la revendication 8 ou 9, d'un récepteur selon l'une quelconque des revendications 1 à 4, dans un test de criblage pour identifier des nouveaux médicaments.

10 11. Procédé pour identifier des ligands fonctionnels pour un récepteur selon l'une quelconque des revendications 1 à 4, ledit procédé comprenant les étapes de

(a) introduction dans une cellule hôte appropriée:

(i) d'un ADN selon l'une quelconque des revendications 5 ou 6, et

15 (ii) d'un gène indicateur approprié lié fonctionnellement à un élément de réponse pour la fonction hormonale (HRE), ledit HRE pouvant être activé par le domaine de liaison d'ADN du récepteur codé par ledit ADN ;

(b) mise en contact de ladite cellule hôte de (a) avec des ligands potentiels qui sont susceptibles de se lier au domaine de liaison du ligand du récepteur codé par ledit ADN de l'étape (a) ; et

20 (c) suivi de l'expression du récepteur codé par ledit gène indicateur de l'étape (a).

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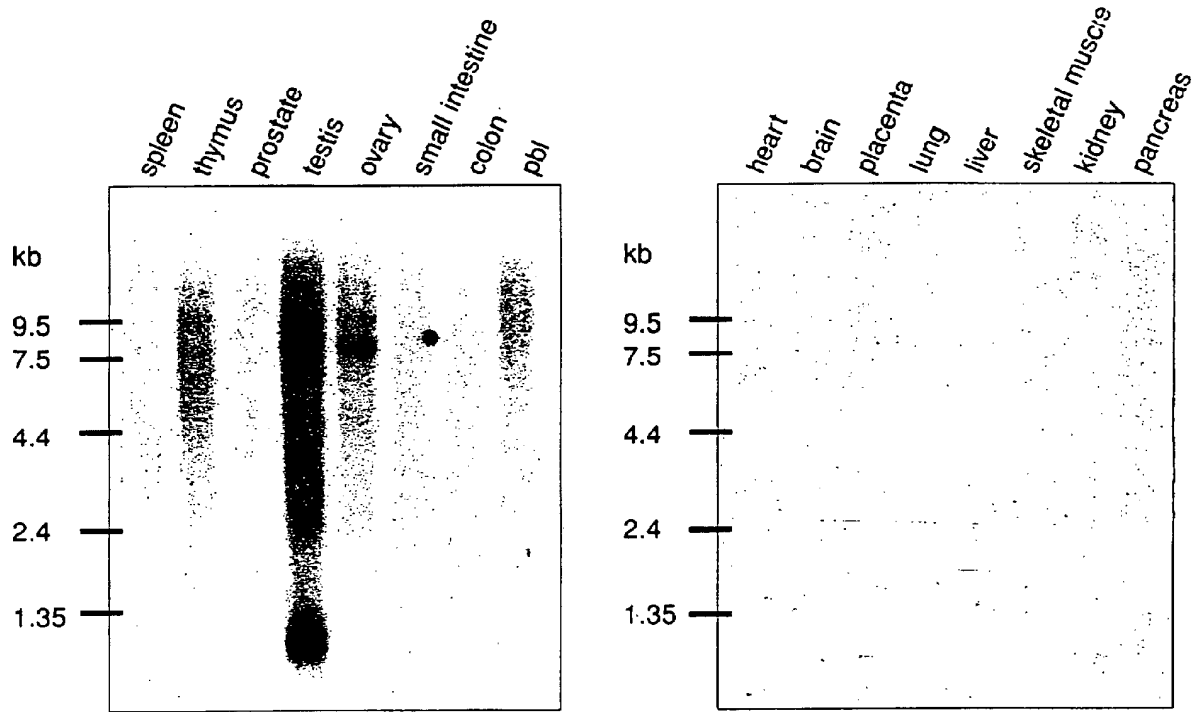


Figure 1

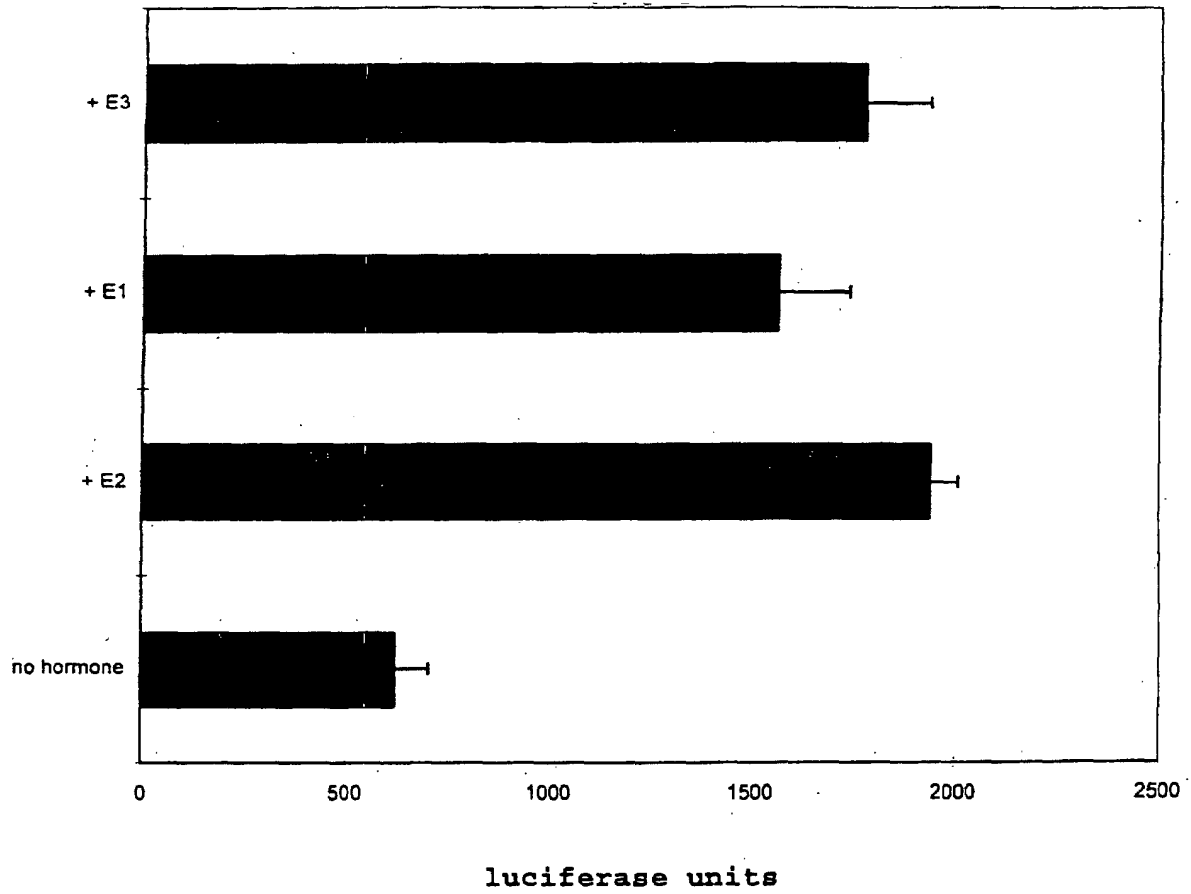


Fig. 2

Transient transfection of CHO cells with Estrogen Receptors Alpha and Beta
Incubation with Estradiol and ICI

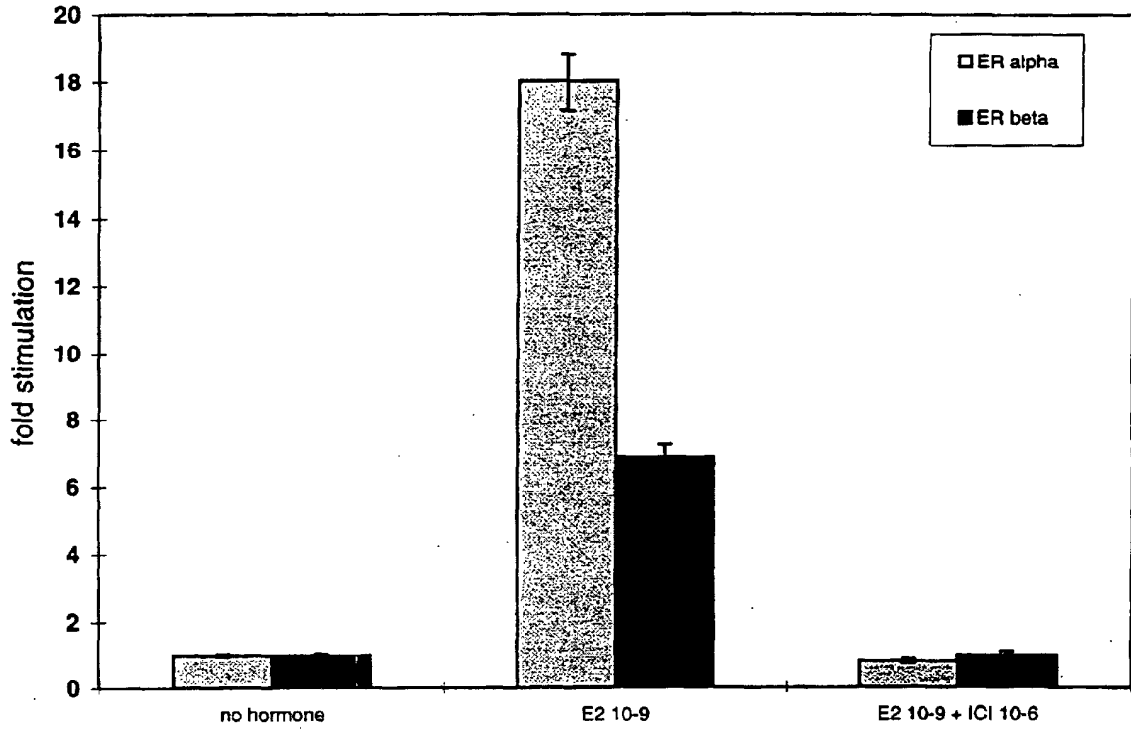


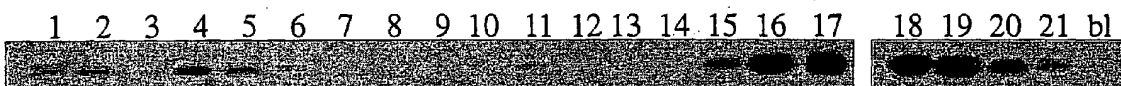
Figure 3

ER α and ER β RT PCR on tissue-representative cell lines

ER α



ER β



- | | | | | |
|-------------|-----------|-------------|--------------|-----------|
| 1. Ishikawa | 6. HOS | 11. Hs-760T | 16. HUV-EC-C | 21. RASMC |
| 2. HEC-1A | 7. U2-OS | 12. SW-954 | 17. BAEC-1 | bl. blank |
| 3. RL95-2 | 8. MG-63 | 13. Hep- G2 | 18. A10 | |
| 4. ECC-1 | 9. MCF-7 | 14. CaCo | 19. A7R5 | |
| 5. SaOS-2 | 10. T47-D | 15. HISM | 20. CavaSMC | |

Figure 4

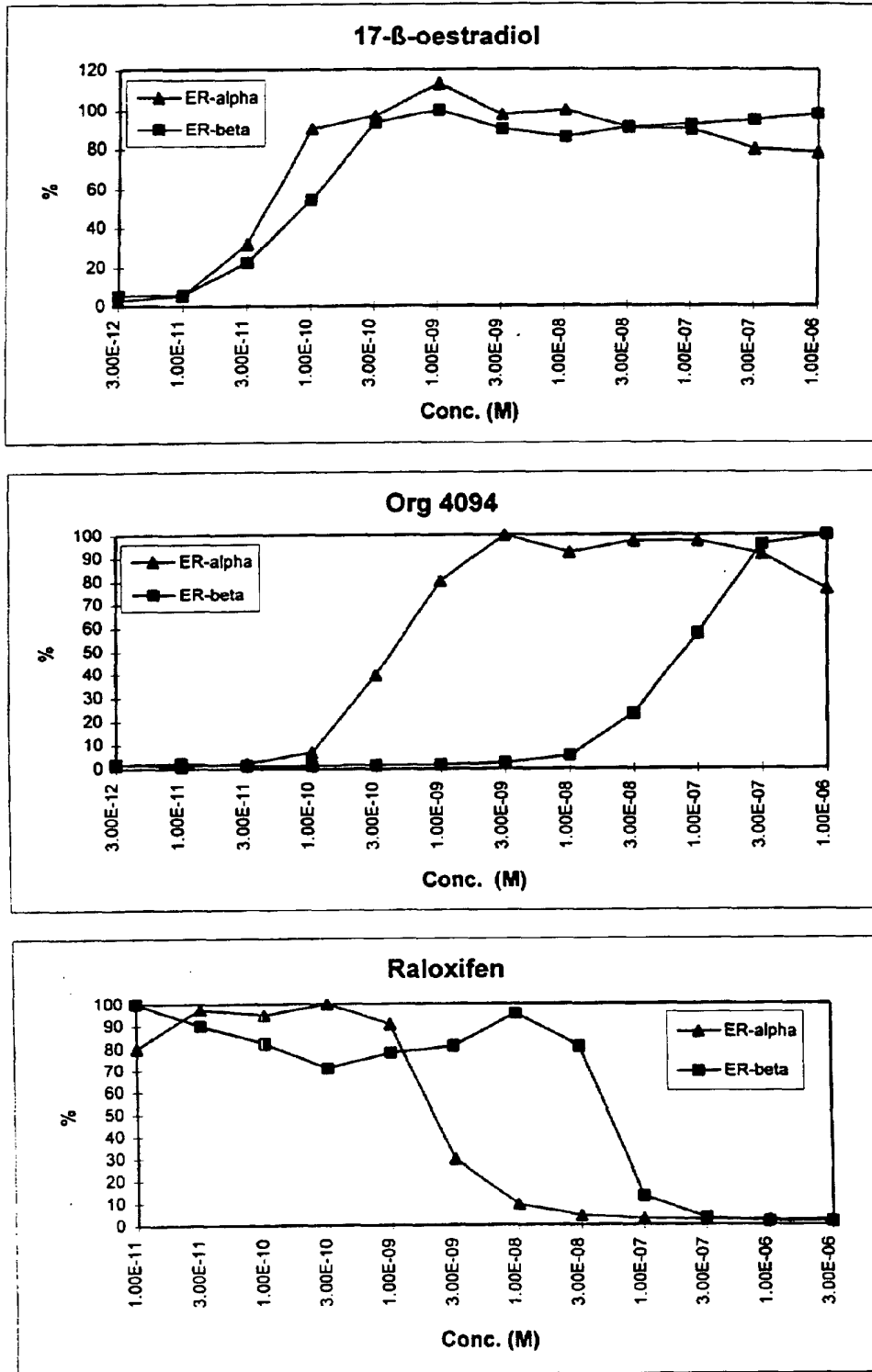


Figure 5